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Assessing changes in ecosystem functioning and services via functional traits of an estuarine fish assemblage

Mestrado em Ecologia Marinha

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“Look deep into nature, and then you will understand everything better.”

Albert Einstein

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Abstract

Biodiversity loss is a pressing global issue. Yet, there is limited knowledge on the consequences of species loss for ecosystem functioning and services. The main objective of this study was to assess the potential influence on the provision of ecosystem services exerted by an estuarine fish assemblage via functional traits, and possible temporal and spatial variations.

To explore links between fish biodiversity and ecosystem functioning and services we reviewed the literature for evidence on relationships between fish functional traits (e.g. body size, diet) and ecosystem service provision to humankind (e.g. nutrient cycling and biological control). Additionally, we used a time-series data on an estuarine fish assemblage (Mondego estuary, Portugal, sampled between 2003 and 2013) as a practical case-study for further developing our approach. For each fish species we assembled data on five functional traits and identified functional groups of species (e.g. through hierarchical cluster analysis). We characterized the potential contribution of the Mondego fish assemblage to the functioning of the ecosystem and its service provision. In addition, we determined the degree of functional redundancy in the fish assemblage, towards an evaluation of the degree of resilience of this assemblage in contributing to ecosystem functioning and service provision.

The most important functional traits identified in the literature review to regulating services were feeding mode, diet and body size and their effects depended on both service and ecosystem. We identified seven functional groups in the fish assemblage, with potential to contribute to all regulating services analysed, except air purification, namely: nutrient cycling, coastal protection and disturbance prevention, maintenance of sediment processes and sediment redistribution, biological control, climate regulation, waste treatment and assimilation and regulation of linkages between ecosystems. However, this contribution was not equal for all groups, as each one was linked to specific services and differently represented in the fish assemblage. Also, fish functional groups showed spatial and inter-annual variation, which is probably associated with environmental conditions. The majority of fish functional traits measures were significantly correlated with salinity, temperature and oxygen and showed strong spatial and annual variation, despite a weak seasonal variation. Functional redundancy of the fish assemblage showed spatial and seasonal variations, which may be related with differences in abundance of species between stations, probably due to different environmental conditions, and seasons, which influence recruitment periods of some species. The annual functional redundancy differed between groups, with group 7 showing null values, possibly being the most vulnerable group to disturbance, and groups 5 and 6 showing the highest values, which may mean they contribute in a more resilient manner to the the provision of ecosystem services such as waste treatment and assimilation and climate regulation.

This study is the first one to assess the provision of ecosystem services by the fish assemblage of the Mondego estuary through their functional traits and estimate their resilience. Future studies should develop the quantification of ecosystem services provided by fish in ecological and economical perspectives, assess the impacts of anthropogenic pressures in functional composition of fish assemblages and thus in the ecosystem services they can provide, and also estimate the resilience of those fish assemblages, which is essential to prioritize conservation efforts of fish assemblages and safeguard the services they provide.

Keywords: fish assemblage; estuaries; ecosystem services; functional traits; resilience

Resumo

A perda de biodiversidade é um problema urgente à escala global. Contudo, o conhecimento sobre as consequências da perda de espécies para o funcionamento e serviços dos ecossistemas é limitado. O principal objectivo deste estudo foi avaliar a potencial influência na provisão de serviços de ecossistema exercida por uma associação de peixes estuarinos através das características funcionais, e as suas possíveis variações temporais e espaciais.

Para explorar as ligações entre a biodiversidade de peixes e o funcionamento e serviços de ecossistema, fizemos uma revisão da literatura para encontrar evidências de relações entre características funcionais de peixes (e.g. tamanho do corpo, dieta) e o funcionamento de ecossistemas/provisão de serviços à humanidade (e. g. reciclagem de nutrientes e controlo biológico). Adicionalmente, usámos uma série cronológica da composição taxonómica de uma associação de peixes estuarina (estuário do Mondego, Portugal, amostrada entre 2003 e 2013) como caso prático de estudo para desenvolver a nossa abordagem. Para cada espécie de peixe, reunimos dados acerca de cinco características funcionais e identificámos grupos funcionais de espécies (e.g. através de análise hierárquica de agrupamento). Caracterizámos a contribuição da associação de peixes do Mondego para o funcionamento do ecossistema e a sua provisão de serviços. Além disso, determinámos o nível de redundância funcional da associação de peixes, para uma avaliação do grau de resiliência desta associação ao contribuir para o funcionamento do ecossistema e provisão de serviços.

As características funcionais mais importantes identificadas na revisão de literatura para os serviços de regulação foram o modo de alimentação, a dieta e o tamanho do corpo e os seus efeitos dependem do serviço e do ecossistema. Identificámos sete grupos funcionais na associação de peixes, com potencial para contribuir para todos os serviços reguladores de ecossistema, excepto purificação do ar, nomeadamente: reciclagem de nutrientes, protecção da costa e prevenção de perturbações, manutenção de processos sedimentares e de redistribuição sedimentar, controlo biológico, regulação do clima, tratamento e assimilação de desperdícios e regulação de ligações entre ecossistemas. No entanto, esta contribuição não foi igual para todos os grupos, já que cada um está ligado a serviços específicos e diferentemente representado na associação de peixes. Além disso, os grupos funcionais de peixes mostraram variação espacial e inter-anual, o que está provavelmente associado a condições ambientais. A maioria das medidas de características funcionais das espécies foram significativamente correlacionadas com a salinidade, temperatura e oxigénio e mostraram uma forte variação espacial e anual, apesar de uma fraca variação sazonal. A redundância funcional da associação de peixes mostrou variação espacial e sazonal, o que pode estar relacionado com diferenças na abundância de espécies entre estações de amostragem, provavelmente devido a diferentes condições ambientais e estações do ano, que influenciam os períodos de recrutamento de algumas espécies. A redundância funcional média anual variou entre grupos, com o grupo 7 a mostrar valores nulos, sendo possivelmente o grupo mais vulnerável a perturbações e os grupos 5 e 6 a mostrar os valores mais altos, o que pode significar que eles contribuem de uma maneira mais resiliente para a provisão de serviços de ecossistema como o tratamento e assimilação de desperdícios e a regulação do clima.

Este estudo foi o primeiro a avaliar a provisão de serviços de ecossistema relacionados com a associação de peixes do estuário do Mondego através das suas características funcionais e a estimar a sua resiliência. Estudos futuros deverão desenvolver a quantificação de serviços de ecossistema fornecidos por

peixes do ponto de vista ecológico e económico, avaliar os impactos das pressões antropogénicas na composição funcional de associações de peixes e nos serviços de ecossistema que elas conseguem fornecer, e também estimar a resiliência dessas associações de peixes, a qual é essencial para prioritizar esforços de conservação em associações de peixes e salvaguardar os serviços que elas fornecem.

Palavras-chave: associação de peixes; estuários; serviços de ecossistema; características funcionais; resiliência

Resumo alargado

A biodiversidade de organismos a todos os níveis (taxonómico, funcional e genético) é essencial para o ser humano, fornecendo recursos naturais e afectando processos ecológicos importantes (e.g. produção de biomassa e fotossíntese), que controlam os fluxos de nutrientes, energia e matéria orgânica pelo ambiente, além de mediarem o fornecimento de serviços de ecossistema. Os serviços de ecossistema são as contribuições directas e indirectas dos ecossistemas para o bem-estar humano e podem ser de vários tipos, conforme a classificação usada – de acordo com a Economia dos Ecossistemas e Biodiversidade são: provisão, regulação, cultural e de habitat. A biodiversidade tem um papel fundamental no fornecimento de serviços de ecossistema, e especialmente as características funcionais dos organismos, isto é, as que influenciam directamente o seu desempenho e são mensuráveis ao nível individual (e.g. tamanho do corpo e mobilidade) são muito úteis para descrever a contribuição das espécies para o funcionamento dos ecossistemas e para a entrega de serviços de ecossistema.

A sobre-exploração de recursos naturais pelo ser humano, bem como outras actividades antropogénicas, tem levado à degradação dos ecossistemas e a alterações na estrutura e composição das comunidades biológicas, o que afecta o funcionamento dos ecossistemas e diminui a sua capacidade de fornecimento de serviços. Assim, torna-se necessário compreender até que ponto é que os ecossistemas podem absorver perturbações e conseguir manter as suas características essenciais, capacidade esta a que se dá o nome de resiliência.

Os estuários são sistemas de transição entre água doce e salgada, que constituem importantes habitats para espécies de peixes, oferecendo refúgio de predadores, áreas de viveiro e pontos de passagem para migrações, além de zonas de reprodução e alimentação. Em geral, os sistemas estuarinos apresentam flutuações nas suas condições ambientais, o que leva a que as comunidades que neles habitam apresentem uma certa capacidade de adaptação. Contudo, por apresentarem elevada produtividade, são também intensamente explorados pelas populações humanas, o que tem vindo a comprometer a sua integridade e a alterar as suas comunidades biológicas.

Este estudo pretendeu avaliar a influência na provisão de serviços de ecossistema exercida por uma associação de peixes de um estuário através das suas características funcionais, bem como a sua variação espacial e temporal. Como caso de estudo, considerou-se a associação de peixes do estuário do Mondego, a qual tem sido monitorizada há mais de uma década. Especificamente, os objectivos foram: obter informação sobre ligações entre características funcionais de peixes e a provisão de serviços de ecossistema (com base numa revisão de literatura); identificar grupos funcionais de peixes dentro do estuário do Mondego (grupos de espécies com características funcionais semelhantes) e a sua possível contribuição para a provisão de serviços reguladores de ecossistema; investigar padrões de variação das características funcionais dos peixes e dos grupos funcionais e sua relação com os factores ambientais subjacentes; explorar a resiliência dos serviços de ecossistema fornecidos pela associação de peixes através do cálculo da redundância funcional (número de espécies que desempenham funções semelhantes).

A revisão de literatura permitiu identificar ligações entre características funcionais de peixes e a provisão de serviços reguladores de ecossistema, embora a maioria das ligações fosse indirecta. As características funcionais com maior número de ligações a este tipo de serviços foram o modo de alimentação, a dieta e o tamanho do corpo. Os serviços reguladores de ecossistema com maior número de ligações a características funcionais foram a reciclagem de nutrientes, a protecção costeira e prevenção de perturbações, o controlo biológico e a regulação de ligações entre ecossistemas. Após a caracterização

funcional da associação de peixes em estudo, tendo em conta cinco características funcionais (mobilidade, tamanho do corpo, dieta, modo de aquisição do alimento e preferência de salinidade), bem como uma análise hierárquica de agrupamento, foram identificados sete grupos funcionais de peixes no estuário do Mondego, com capacidade para fornecer diferentes serviços reguladores de ecossistema: reciclagem de nutrientes, controlo biológico, manutenção de processos sedimentares e de redistribuição de sedimentos, protecção costeira e prevenção de perturbações, regulação do clima, tratamento e assimilação de desperdícios e regulação de ligações entre ecossistemas. No estuário do Mondego, a comunidade de peixes parece influenciar de forma mais importante os seguintes serviços de ecossistema: a reciclagem de nutrientes, o controlo biológico, a regulação do clima e o tratamento e assimilação de desperdícios. Para todas as análises relativas à associação de peixes e grupos funcionais, foram consideradas três medidas: biomassa, densidade e número de espécies. As variações dos grupos funcionais observadas na análise foram sobretudo temporais e espaciais, o que estará provavelmente relacionado com alterações nos padrões climatéricos ao longo do período de amostragem (no primeiro caso) e com diferentes condições ambientais nos locais de amostragem, sobretudo em relação à salinidade, temperatura e oxigénio (no segundo caso). No entanto, também foram observadas variações sazonais para alguns grupos funcionais, ainda que menores, que podem estar relacionadas com a temperatura e períodos de recrutamento, já que estes grupos contêm espécies marinhas migradoras e que utilizam o estuário como zona de viveiro.

A relação entre as características funcionais e as variáveis ambientais (salinidade, temperatura, percentagem de oxigénio dissolvido, precipitação, escoamento e índice NAO) foi explorada através de modelos lineares generalizados e modelos de partição hierárquica de variância. Estas análises evidenciaram uma pequena influência significativa da salinidade, temperatura e oxigénio e especialmente uma elevada percentagem de variação explicada sobretudo pelo local e ano. Para a maioria das características funcionais, o ano de 2003 foi o ano com valores mais elevados e o ano de 2006 foi o ano em que se registaram valores mais baixos - o que poderá estar relacionado com a baixa salinidade (devida a forte precipitação) e com o facto de a maioria das espécies do estuário do Mondego serem marinhas.

Finalmente, foi avaliada a redundância funcional desta associação de peixes com o objectivo de avaliar a resiliência da influência desta comunidade no fornecimento de serviços reguladores de ecossistema. A redundância funcional da associação de peixes em estudo variou sazonal e espacialmente, com valores mais elevados na primavera e no local de amostragem mais a montante. Em relação aos sete grupos funcionais desta associação, a redundância funcional (média anual) foi mais elevada para os grupos 5 e 6 (os que apresentam maior densidade, biomassa e riqueza) e mais baixa para os grupos 1 e 4, com o grupo 7 a mostrar valores nulos, o que poderá significar que é o grupo menos resiliente e cuja provisão de serviços será mais afectada com a perturbação e perda de espécies. Assim, os serviços de ecossistema mais resilientes nesta associação de peixes serão os fornecidos pelos grupos 5 e 6, nomeadamente: tratamento e assimilação de desperdícios, regulação do clima, reciclagem de nutrientes e controlo biológico. No entanto, o grupo 6 também tem capacidade para fornecer outros serviços de ecossistema, como a protecção costeira e prevenção de perturbações, manutenção de processos sedimentares e redistribuição de sedimentos e regulação de ligações entre ecossistemas, o que pode aumentar a resiliência destes serviços. No entanto, isto depende do funcionamento do ecossistema, o qual é condicionado por múltiplas variáveis, incluindo a resposta das espécies à perturbação e as interacções entre espécies, aspectos estes que deverão ser investigados em estudos futuros.

Este estudo constitui uma abordagem ao estudo da relação entre as características funcionais de uma comunidade de peixes estuarinos e o fornecimento dos serviços de ecossistema que poderá ser facilmente aplicada a outros estuários e constitui um passo importante para a compreensão da influência da

biodiversidade sobre o bem-estar humano. Além disso, ao avaliar a redundância funcional para esta associação de peixes, este estudo permitiu estimar o seu grau de resiliência, o que é importante para a sua conservação e para a manutenção do funcionamento deste ecossistema. A estimativa do grau de resiliência distinguiu os serviços de ecossistema mais importantes no estuário e a capacidade de manutenção de serviços pelos grupos funcionais da associação de peixes estudada. Os grupos funcionais com maiores valores de biomassa, densidade e número de espécies mostraram ser os mais resilientes na provisão de serviços de ecossistema, o que mostra a influência da quantidade de espécies para a redundância funcional e consequentemente para a resiliência de serviços de ecossistema.

No futuro, deverão ser realizados mais estudos sobre ligações entre peixes e serviços de ecossistema (especialmente porque este grupo biológico é muito afectado pela pesca e por alterações ambientais) nomeadamente para o desenvolvimento de outras formas de quantificação directa de serviços de ecossistema fornecidos por peixes. É ainda importante investigar directamente os efeitos de pressões antropogénicas na composição funcional das comunidades de peixes e consequentemente no funcionamento dos ecossistemas.

Palavras-chave: associação de peixes; estuários; serviços de ecossistema; características funcionais; resiliência

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CHAPTER 1

General introduction

General introduction

Biodiversity is currently understood as the "variability among living organisms and their habitats from all sources, including diversity within species, between species and within entire ecosystems" (Pinto *et al.* 2014) and considering all components – taxonomic, functional and phylogenetic diversity (Purschke *et al.* 2013). It is crucial to human welfare, both directly and indirectly, as it provides natural resources (e.g. food and drinkable water) and also affects important ecosystem functions (i. e. ecological processes that control the fluxes of energy, nutrients and organic matter through the environment; Cardinale *et al.* 2012), such as biomass production and soil formation, which mediate the provision of ecosystem services (Daily 1997; Díaz *et al.* 2006).

Ecosystem services (ES) constitute "the direct and indirect contributions of ecosystems to human well-being" (de Groot *et al.* 2010) and are essential to human survival (Kremen 2005). It is therefore necessary to classify them in a comprehensive way (Atkins *et al.* 2011). According to the Millennium Ecosystem Assessment (2005), there are four types of ecosystem services: provisioning (e.g. food and drinkable water), regulating (e.g. climate regulation and water purification), cultural (e.g. aesthetic and recreational values) and supporting (e.g. nutrient cycling and primary production). However, this classification has been challenged, for example, for considering supporting services as directly beneficial to humans, when they actually constitute a basic support to the provision of other services (Böhnke-Henrichs et al. 2013). Since then, other classifications have been developed (Beaumont *et al.* 2007; Fisher *et al.* 2009; de Groot *et al.* 2010; Atkins *et al.* 2011; Böhnke-Henrichs *et al.* 2013; Liquete *et al.* 2013; Hattam *et al.* 2015), which have gained more consensus, including the one from de Groot et al. (2010), which classifies ecosystem services in four categories: provisioning, regulating, cultural and habitat. This last category includes the services of life cycle maintenance of migratory species and gene pool protection, which are dependent of the state of the habitat (de Groot et al. 2010). There has been an evolution of knowledge about ecosystem services in recent decades (Farber *et al.* 2006; Worm *et al.* 2006; Boyd & Banzhaf 2007; Wallace 2007; Liquete *et al.* 2013), which is important for conservation and management purposes (Costanza *et al.* 1997).

Several approaches have been developed to clarify the relationship between biodiversity and ecosystem services, exploring the contribution of habitats (Chan *et al.* 2006) and the roles of individual species and groups of species in ecosystem functioning (Hooper *et al.* 2005; Díaz *et al.* 2007; Luck *et al.* 2009; Harrison *et al.* 2014). Moreover, according to Luck *et al.* (2009), it is necessary to identify the organisms and their characteristics that are linked with the provision of services and also determine how changes in these organisms affect those services. In this sense, functional traits (i.e. characteristics of organisms directly influencing the organism's performance, for example body size and mobility; Mouillot *et al.* 2012) are useful predictors to describe links between species, ecosystem functioning and ecosystem service delivery (Lavorel & Garnier 2002; Díaz *et al.* 2004; Hooper *et al.* 2005; de Bello *et al.* 2010; Harrison *et al.* 2014; Gagic *et al.* 2015). These linkages have been predominantly studied for some groups of organisms such as plants, for which there is abundant evidence of associations between traits and services (de Bello *et al.* 2010).

There has been a considerable increase in the number of studies investigating the complex role of biodiversity (chiefly species richness) on ecosystem functioning (Worm *et al.* 2006; Lefcheck *et al.* 2015), as well as regarding the influence of species traits in the functional structure of ecosystems (de Bello *et al.*

2010; Cardinale *et al.* 2012; Gagic *et al.* 2015) and on ecosystem functioning and the provision of ecosystem services (Balvanera *et al.* 2006; Harrison *et al.* 2014). Some studies developed the concept of functional groups – groups of species with similar functional traits – which is useful in the context of evaluating the provision of ecosystem services since groups can be defined based on functional traits that are important to services of interest (Lavorel *et al.* 1997; McGrady-Steed *et al.* 1997; Gerino *et al.* 2003; Luck *et al.* 2009; García-Llorente *et al.* 2011). For example, García-Llorente *et al.* (2011) showed the potential contribution of each functional group of aquatic plants to the delivery of ecosystem services such as provisioning of medicine resources, aesthetic values, consumption by livestock, and water filtration. Also, Gerino *et al.* (2003) assigned macro-invertebrates into functional groups considering different types of aquatic sediments, which contributed to a better understanding of ecological processes such as bioturbation (i.e. "reworking and mixing of sediment at the sediment-water interface, accomplished by activities of benthic organisms, which alter the structure and properties of sediment and influence the transport of solutes and particulate matter"; Adámek & Maršálek 2013) which mediates the maintenance of sediment processes, according to Holmlund and Hammer (1999).

The increasing degradation of ecosystems due to anthropogenic activities and over-exploitation of natural resources lead to alterations in the structure and composition of biological communities (Kremen 2005; Díaz *et al.* 2006). Moreover, these human-induced alterations can originate loss of key species that perform particular functions on which important ecosystem processes such as bioturbation and primary productivity (Solan *et al.* 2004) depend, which can decrease the capacity of ecosystems to provide essential services (Mouillot *et al.* 2013; Pratchett *et al.* 2014). Thus, it is necessary to estimate the capacity of ecosystems to absorb disturbance and retain their essential characteristics - meaning estimating their ecological resilience (Holling 1973). Elmqvist *et al.* (2003) emphasizes the role of biodiversity for ecosystem resilience in the face of undergoing change, as also referred by Walker (1992) and Norberg *et al.* (2001). Moreover, it is currently widely accepted that, in addition to taxonomic-based diversity indices, functional and phylogenetic diversity should be studied in order to assess ecosystem resilience (Meynard *et al.* 2011; Gagic *et al.* 2015). In this context, different approaches have been developed, for example, suggesting that resilience of ecosystems depends on the variety and distribution of functional groups (Peterson *et al.* 1998; Gunderson 2000) and on diversity of species within these groups (Luck *et al.* 2003). Furthermore, Mouillot *et al.* (2014) highlighted the role of functional redundancy (i.e. the number of different species that perform similar ecological functions; Micheli & Halpern 2005) in preserving ecosystem functioning after decrease of species diversity. Also, the response of species with similar functions to environmental variation – called "response diversity" - is also crucial to ecosystem resilience (Elmqvist *et al.* 2003; Folke *et al.* 2004), because if all these species are equally vulnerable to disturbance, functional redundancy is not sufficient to maintain ecosystem functioning (Hughes *et al.* 2005; Muntadas *et al.* 2016). For instance, rare species may have an important role in this process, since they can share similar functional traits with common species but may respond differently to stress (Boero 1994; Walker *et al.* 1999; Lyons *et al.* 2005; Mouillot *et al.* 2013). Although it is still difficult to measure resilience, certain indicators can be used to estimate it, such as functional diversity (Peterson *et al.* 1998; Elmqvist *et al.* 2003), functional redundancy (de Bello *et al.* 2007; van der Linden *et al.* 2012; Mouillot *et al.* 2014; Muntadas *et al.* 2016) and functional vulnerability (i.e. potential decrease of functional diversity after species loss; Mouillot *et al.* 2014). Moreover, it is important to consider the capacity of adaptation by species, which can change their functional roles in different environmental contexts (Wellnitz & Poff 2001).

Estuaries are transitional systems that are extremely productive and valuable for human society, being highly explored (Costanza *et al.* 1997; Kennish 2002; Barbier *et al.* 2011). Also, they are significantly important for fish communities, since they provide reproduction habitats, nursery grounds, migratory routes and protection from predators (Beck *et al.* 2001; Martinho *et al.* 2008; Baptista *et al.* 2013; Fonseca *et al.* 2013) being essential to the maintenance and regeneration of numerous fishery resources (Nicolas *et al.* 2007; Vasconcelos *et al.* 2011). However, these systems are subject to high anthropogenic pressure and environmental alterations, which are increasingly deteriorating their integrity and also affecting important ecological processes, such as biogeochemical cycling and flux regulation of water and pollutants (Kennish 2002; Borja *et al.* 2010; Barbier *et al.* 2011). The loss of large predators and herbivorous species, habitats for reproduction and filtering capacity are some of the changes in structure and functioning that result from over-exploitation and degradation of estuarine systems (Lotze *et al.* 2006), and that affect the delivery of critical ecosystem services, such as sustainable fisheries, provision of nursery habitats, cleansing processes and shoreline protection (Worm *et al.* 2006; Barbier *et al.* 2011; Rodrigues & Pardal 2015). Therefore, the conservation of these habitats is critical in ecosystem management (Beck *et al.* 2001; Nicolas *et al.* 2007).

Fishes are a key component of biodiversity in estuaries. They display a wide range of functional traits and behaviors, being involved in numerous ecological processes (Villéger *et al.* 2012). Besides, fishes can be considered relevant ecological indicators of estuarine conditions at various scales (Whitfield & Elliott 2002; Nicolas *et al.* 2010). In these systems, fish communities include estuarine resident species, marine and freshwater species that come into estuaries adventitiously or as migrants, and also diadromous and amphidromous species (Elliott *et al.* 2007; Vasconcelos *et al.* 2015). The structure of fish communities can be considered as being shaped by geographical localization as well as by a set of environmental variables including salinity and temperature (Whitfield & Elliott 2002; Vasconcelos *et al.* 2015; Henriques *et al.* 2016), but the main driver influencing the assemblages´s structure within estuaries is their longitudinal salinity gradient (Whitfield *et al.* 2012; Vasconcelos *et al.* 2015).

The main goal of this study is to assess the potential provision of ecosystem services by an estuarine fish assemblage (by characterizing its functional traits) and also possible temporal variations using the Mondego estuary as a case study since it has been monitored systematically throughout the last decade. This approach should improve the understanding about the importance of fish in regulating ecosystem functions and in generating ecosystem services derived from these transitional systems. The study ultimately aims to contribute to enable the protection and sustainable management of estuaries in a world increasingly impacted by Humans especially in coastal areas.

Literature cited

Adámek Z. & Maršálek B. (2013). Bioturbation of sediments by benthic macroinvertebrates and fish and its implication for pond ecosystems: a review. *Aquaculture International*, 21: 1-17.

Atkins J.P., Burdon D., Elliott M. & Gregory A.J. (2011). Management of the marine environment: integrating ecosystem services and societal benefits with the DPSIR framework in a systems approach. *Marine Pollution Bulletin*, 62: 215-226.

Balvanera P., Pfisterer A.B., Buchmann N., He J.S., Nakashizuka T., Raffaelli D. & Schmid B. (2006). Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecology Letters*, 9: 1146-1156.

Baptista J., Pato P., Pereira E., Duarte A.C. & Pardal M.A. (2013). PCBs in the fish assemblage of a southern European estuary. *Journal of Sea Research*, 76: 22-30.

Barbier E.B., Hacker S.D., Kennedy C., Koch E.W., Stier A.C. & Silliman B.R. (2011). The value of estuarine and coastal ecosystem services. *Ecological Monographs*, 81: 169-193.

Beaumont N.J., Austen M.C., Atkins J.P., Burdon D., Degraer S., Dentinho T.P., Derous S., Holm P., Horton T., van Ierland E., Marboe A.H., Starkey D.J., Townsend M. & Zarzycki T. (2007). Identification, definition and quantification of goods and services provided by marine biodiversity: implications for the ecosystem approach. *Marine Pollution Bulletin*, 54: 253-265.

Beck M.W., Heck K.L., Able K.W., Childers D.L., Eggleston D.B., Gillanders B., Halpern B., Hays C.G., Hoshino K., Minello T.J., Orth R.J., Sheridan P.F. & Weinstein M. (2001). The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *BioScience*, 51: 633-641.

Boero F. (1994). Fluctuations and variations in coastal marine environments. *Marine Ecology-Pubblicazioni Della Stazione Zoologica Di Napoli I*, 15: 3-25.

Böhnke-Henrichs A., Baulcomb C., Koss R., Hussain S.S. & de Groot R.S. (2013). Typology and indicators of ecosystem services for marine spatial planning and management. *Journal of Environmental Management*, 130: 135-145.

Borja A., Dauer D.M., Elliott M. & Simenstad C.A. (2010). Medium- and long-term recovery of estuarine and coastal ecosystems: patterns, rates and restoration effectiveness. *Estuaries and Coasts*, 33: 1249-1260.

Boyd J. & Banzhaf S. (2007). What are ecosystem services? The need for standardized environmental accounting units. *Ecological Economics*, 63: 616-626.

Cardinale B.J., Duffy J.E., Gonzalez A., Hooper D.U., Perrings C., Venail P., Narwani A., Mace G.M., Tilman D., Wardle D.A., Kinzig A.P., Daily G.C., Loreau M., Grace J.B., Larigauderie A., Srivastava D.S. & Naeem S. (2012). Biodiversity loss and its impact on humanity. *Nature*, 486: 59-67.

Chan K.M., Shaw M.R., Cameron D.R., Underwood E.C. & Daily G.C. (2006). Conservation planning for ecosystem services. *PLoS Biology*, 4: 2138-2152.

Costanza R., dArge R., deGroot R., Farber S., Grasso M., Hannon B., Limburg K., Naeem S., ONeill R.V., Paruelo J., Raskin R.G., Sutton P. & vandenBelt M. (1997). The value of the world's ecosystem services and natural capital. *Nature*, 387: 253-260.

Daily G.C. (1997). *Nature's services: societal dependence on natural ecosystems*, Washington.

de Bello F., Lavorel S., Díaz S., Harrington R., Cornelissen J.H.C., Bardgett R.D., Berg M.P., Cipriotti P., Feld C.K., Hering D., Martins da Silva P., Potts S.G., Sandin L., Sousa J.P., Storkey J., Wardle D.A. & Harrison P.A. (2010). Towards an assessment of multiple ecosystem processes and services via functional traits. *Biodiversity and Conservation*, 19: 2873-2893.

de Bello F., Leps J., Lavorel S. & Moretti M. (2007). Importance of species abundance for assessment of trait composition: an example based on pollinator communities. *Community Ecology*, 8: 163-170.

de Groot R., Fisher B. & Christie M. (2010). Integrating the ecological and economic dimensions in biodiversity and ecosystem service valuation. In: *The Economics of Ecosystems and Biodiversity: The Ecological and Economic Foundations* (ed. Kadekodi GK), pp. 2 - 40.

Díaz S., Fargione J., Chapin F.S., 3rd & Tilman D. (2006). Biodiversity loss threatens human well-being. *PLoS Biology*, 4: 1300-1305.

Díaz S., Hodgson J.G., Thompson K., Cabido M., Cornelissen J.H.C., Jalili A., Montserrat-Marti G., Grime J.P., Zarrinkamar F., Asri Y., Band S.R., Basconcelo S., Castro-Diez P., Funes G., Hamzehee B.,

Khoshnevi M., Perez-Harguindeguy N., Perez-Rontome M.C., Shirvany F.A., Vendramini F., Yazdani S., Abbas-Azimi R., Bogaard A., Boustani S., Charles M., Dehghan M., de Torres-Espuny L., Falczuk V., Guerrero-Campo J., Hynd A., Jones G., Kowsary E., Kazemi-Saeed F., Maestro-Martinez M., Romo-Diez A., Shaw S., Siavash B., Villar-Salvador P. & Zak M.R. (2004). The plant traits that drive ecosystems: Evidence from three continents. *Journal of Vegetation Science*, 15: 295-304.

Díaz S., Lavorel S., de Bello F., Quetier F., Grigulis K. & Robson T.M. (2007). Incorporating plant functional diversity effects in ecosystem service assessments. *Proceedings of the National Academy of Sciences of the United States of America*, 104: 20684-20689.

Elliott M., Whitfield A.K., Potter I.C., Blaber J.M., Cyrus D.P., Nordlie F.G. & Harrison T.D. (2007). The guild approach to categorizing estuarine fish assemblages: a global review. *Fish and Fisheries*, 8: 241-268.

Elmqvist T., Folke C., Nyström M., Peterson G., Bengtsson J., Walker B. & Norberg J. (2003). Response diversity, ecosystem change, and resilience. *Frontiers in Ecology and the Environment*, 1: 488-494.

Farber S., Costanza R., Childers D.L., Erickson J., Gross K., Grove M., Hopkinson C.S., Kahn J., Pincetl S., Troy A., Warren P. & Wilson M. (2006). Linking ecology and economics for ecosystem management. *BioScience*, 56: 121-133.

Fisher B., Turner R.K. & Morling P. (2009). Defining and classifying ecosystem services for decision making. *Ecological Economics*, 68: 643-653.

Folke C., Carpenter S., Walker B., Scheffer M., Elmqvist T., Gunderson L. & Holling C.S. (2004). Regime shifts, resilience, and biodiversity in ecosystem management. *Annual Review of Ecology, Evolution, and Systematics*, 35: 557-581.

Fonseca V.F., Vasconcelos R.P., Gamito R., Pasquaud S., Gonçalves C.I., Costa J.L., Costa M.J. & Cabral H.N. (2013). Fish community-based measures of estuarine ecological quality and pressure–impact relationships. *Estuarine, Coastal and Shelf Science*, 134: 128-137.

Gagic V., Bartomeus I., Jonsson T., Taylor A., Winqvist C., Fischer C., Slade E.M., Steffan-Dewenter I., Emmerson M., Potts S.G., Tscharntke T., Weisser W. & Bommarco R. (2015). Functional identity and diversity of animals predict ecosystem functioning better than species-based indices. *Proceedings of the Royal Society B: Biological Sciences*, 282: 1-28.

García-Llorente M., Martín-López B., Díaz S. & Montes C. (2011). Can ecosystem properties be fully translated into service values? An economic valuation of aquatic plant services. *Ecological Applications*, 21: 3083 - 3103.

Gerino M., Stora G., François-Carcaillet F., Gilbert F., Poggiale J.-C., Mermillod-Blondin F., Desrosiers G. & Vervier P. (2003). Macro-invertebrates functional groups in freshwater and marine sediments: a commun mechanistic classification. *Vie et Milieu*: 221-231.

Gunderson L. (2000). Ecological resilience - in theory and application. *Annual Review of Ecology, Evolution, and Systematics*: 425-439.

Harrison P.A., Berry P.M., Simpson G., Haslett J.R., Blicharska M., Bucur M., Dunford R., Egoh B., Garcia-Llorente M., Geamana N., Geertsema W., Lommelen E., Meiresonne L. & Turkelboom F. (2014). Linkages between biodiversity attributes and ecosystem services: A systematic review. *Ecosystem Services*, 9: 191-203.

Hattam C., Atkins J.P., Beaumont N., Borger T., Bohnke-Henrichs A., Burdon D., de Groot R., Hoefnagel E., Nunes P.A.L.D., Piwowarczyk J., Sastre S. & Austen M.C. (2015). Marine ecosystem services: Linking indicators to their classification. *Ecological Indicators*, 49: 61-75.

Henriques S., Cardoso P., Cardoso I., Laborde M., Cabral H.N. & Vasconcelos R.P. (2016). Processes underpinning fish species composition patterns in estuarine ecosystems worldwide. *Journal of Biogeography*, In press.

Holling C.S. (1973). Resilience and stability of ecological systems. *Annual Review of Ecology, Evolution, and Systematics*, 4: 1-23.

Holmlund C.M. & Hammer M. (1999). Ecosystem services generated by fish populations. *Ecological Economics*, 29: 253-268.

Hooper D.U., Chapin F.S., Ewel J.J., Hector A., Inchausti P., Lavorel S., Lawton J.H., Lodge D.M., Loreau M., Naeem S., Schmid B., Setala H., Symstad A.J., Vandermeer J. & Wardle D.A. (2005). Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs*, 75: 3-35.

Hughes T.P., Bellwood D.R., Folke C., Steneck R.S. & Wilson J. (2005). New paradigms for supporting the resilience of marine ecosystems. *Trends in Ecology and Evolution*, 20: 380-386.

Kennish M.J. (2002). Environmental threats and environmental future of estuaries. *Environmental Conservation*, 29: 78-107.

Kremen C. (2005). Managing ecosystem services: what do we need to know about their ecology? *Ecology Letters*, 8: 468-479.

Lavorel S. & Garnier E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology*, 16: 545-556.

Lavorel S., McIntyre S., Landsberg J. & Forbes T.D.A. (1997). Plant functional classifications: from general groups to specific groups based on response to disturbance. *Trends in Ecology and Evolution*, 12: 474-478.

Lefcheck J.S., Byrnes J.E., Isbell F., Gamfeldt L., Griffin J.N., Eisenhauer N., Hensel M.J., Hector A., Cardinale B.J. & Duffy J.E. (2015). Biodiversity enhances ecosystem multifunctionality across trophic levels and habitats. *Nature Communications*, 6: 1-7.

Liquete C., Piroddi C., Drakou E.G., Gurney L., Katsanevakis S., Charef A. & Egoh B. (2013). Current status and future prospects for the assessment of marine and coastal ecosystem services: a systematic review. *PLoS One*, 8: 1-15.

Lotze H.K., Lenihan H.S., Bourque B.J., Bradbury R.H., Cooke R.G., Kay M.C., Kidwell S.M., Kirby M.X., Peterson C.H. & Jackson J.B. (2006). Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science*, 312: 1806-1809.

Luck G.W., Daily G.C. & Ehrlich P.R. (2003). Population diversity and ecosystem services. *Trends in Ecology and Evolution*, 18: 331-336.

Luck G.W., Harrington R., Harrison P.A., Kremen C., Berry P.M., Bugter R., Dawson T.P., de Bello F., Diaz S., Feld C.K., Haslett J.R., Hering D., Kontogianni A., Lavorel S., Rounsevell M., Samways M.J., Sandin L., Settele J., Sykes M.T., van den Hove S., Vandewalle M. & Zobel M. (2009). Quantifying the Contribution of Organisms to the Provision of Ecosystem Services. *BioScience*, 59: 223-235.

Lyons K.G., Brigham C.A., Traut B.H. & Schwartz M.W. (2005). Rare species and ecosystem functioning. *Conservation Biology*, 19: 1019-1024.

Martinho F., Viegas I., Dolbeth M., Leitão R., Cabral H.N. & Pardal M.A. (2008). Assessing estuarine environmental quality using fish-based indices: performance evaluation under climatic instability. *Marine Pollution Bulletin*, 56: 1834-1843.

McGrady-Steed J., Harris P.M. & Morin P.J. (1997). Biodiversity regulates ecosystem predictability. *Nature*, 390: 162-165.

Meynard C.N., Devictor V., Mouillot D., Thuiller W., Jiguet F. & Mouquet N. (2011). Beyond taxonomic diversity patterns: how do a, b and g components of bird functional and phylogenetic diversity respond to environmental gradients across France? *Global Ecology and Biogeography*, 20: 893-903.

Micheli F. & Halpern B.S. (2005). Low functional redundancy in coastal marine assemblages. *Ecology Letters*, 8: 391-400.

Mouillot D., Bellwood D.R., Baraloto C., Chave J., Galzin R., Harmelin-Vivien M., Kulbicki M., Lavergne S., Lavorel S., Mouquet N., Paine C.E., Renaud J. & Thuiller W. (2013). Rare species support vulnerable functions in high-diversity ecosystems. *PLoS Biology*, 11: 1-11.

Mouillot D., Graham N.A., Villeger S., Mason N.W. & Bellwood D.R. (2012). A functional approach reveals community responses to disturbances. *Trends in Ecology and Evolution*, 28: 167-177.

Mouillot D., Villeger S., Parravicini V., Kulbicki M., Arias-Gonzalez J.E., Bender M., Chabanet P., Floeter S.R., Friedlander A., Vigliola L. & Bellwood D.R. (2014). Functional over-redundancy and high functional vulnerability in global fish faunas on tropical reefs. *Proceedings of the National Academy of Sciences of the United States of America*, 111: 13757-13762.

Muntadas A., de Juan S. & Demestre M. (2016). Assessing functional redundancy in chronically trawled benthic communities. *Ecological Indicators*, 61: 882-892.

Nicolas D., Le Loc'h F., Désaunay Y., Hamon D., Blanchet A. & Le Pape O. (2007). Relationships between benthic macrofauna and habitat suitability for juvenile common sole (Solea solea, L.) in the Vilaine estuary (Bay of Biscay, France) nursery ground. *Estuarine, Coastal and Shelf Science*, 73: 639-650.

Nicolas D., Lobry J., Le Pape O. & Boët P. (2010). Functional diversity in European estuaries: Relating the composition of fish assemblages to the abiotic environment. *Estuarine, Coastal and Shelf Science*, 88: 329-338.

Norberg J., Swaney D.P., Dushoff J., Lin J., Casagrandi R. & Levin S.A. (2001). Phenotypic diversity and ecosystem functioning in changing environments: a theoretical framework. *Proceedings of the National Academy of Sciences of the United States of America*, 98: 11376-11381.

Peterson G., Allen C. & Holling C.S. (1998). Ecological resilience, biodiversity, and scale. *Ecosystems*, 1: 6-18.

Pinto R., de Jonge V.N. & Marques J.C. (2014). Linking biodiversity indicators, ecosystem functioning, provision of services and human well-being in estuarine systems: application of a conceptual framework. *Ecological Indicators*, 36: 644-655.

Pratchett M.S., Hoey A.S. & Wilson S.K. (2014). Reef degradation and the loss of critical ecosystem goods and services provided by coral reef fishes. *Current Opinion in Environmental Sustainability*, 7: 37-43.

Purschke O., Schmid B.C., Sykes M.T., Poschlod P., Michalski S.G., Durka W., Kühn I., Winter M., Prentice H.C. & Fridley J. (2013). Contrasting changes in taxonomic, phylogenetic and functional diversity during a long-term succession: insights into assembly processes. *Journal of Ecology*, 101: 857-866.

Rodrigues E.T. & Pardal M.Â. (2015). Primary productivity temporal fluctuations in a nutrient-rich estuary due to climate-driven events. *Estuaries and Coasts*, 38: 1-12.

Solan M., Cardinale B.J., Downing A.L., Engelhardt K.A., Ruesink J.L. & Srivastava D.S. (2004). Extinction and ecosystem function in the marine benthos. *Science*, 306: 1177-1180.

van der Linden P., Patrício J., Marchini A., Cid N., Neto J.M. & Marques J.C. (2012). A biological trait approach to assess the functional composition of subtidal benthic communities in an estuarine ecosystem. *Ecological Indicators*, 20: 121-133.

Vasconcelos R.P., Henriques S., França S., Pasquaud S., Cardoso I., Laborde M. & Cabral H.N. (2015). Global patterns and predictors of fish species richness in estuaries. *Journal of Animal Ecology*, 84: 1331-1341.

Vasconcelos R.P., Reis-Santos P., Costa M.J. & Cabral H.N. (2011). Connectivity between estuaries and marine environment: Integrating metrics to assess estuarine nursery function. *Ecological Indicators*, 11: 1123-1133.

Villéger S., Ramos Miranda J., Flores Hernandez D. & Mouillot D. (2012). Low functional beta-diversity despite high taxonomic beta-diversity among tropical estuarine fish communities. *PLoS One*, 7: 1-10.

Walker B., Kinzig A. & Langridge J. (1999). Plant attribute diversity, resilience, and ecosystem function: the nature and significance of dominant and minor species. *Ecosystems*, 2: 95-113.

Walker B.H. (1992). Biodiversity and ecological redundancy. *Conservation Biology*, 6: 18-23.

Wallace K.J. (2007). Classification of ecosystem services: Problems and solutions. *Biological Conservation*, 139: 235-246.

Wellnitz T. & Poff N.L. (2001). Functional redundancy in heterogeneous environments: implications for conservation. *Ecology Letters*, 4: 177-179.

Whitfield A. & Elliott M. (2002). Fishes as indicators of environmental and ecological changes within estuaries: a review of progress and some suggestions for the future. *Journal of Fish Biology*, 61: 229-250.

Whitfield A.K., Elliott M., Basset A., Blaber S.J.M. & West R.J. (2012). Paradigms in estuarine ecology – A review of the Remane diagram with a suggested revised model for estuaries. *Estuarine, Coastal and Shelf Science*, 97: 78-90.

Worm B., Barbier E.B., Beaumont N., Duffy J.E., Folke C., Halpern B.S., Jackson J.B., Lotze H.K., Micheli F., Palumbi S.R., Sala E., Selkoe K.A., Stachowicz J.J. & Watson R. (2006). Impacts of biodiversity loss on ocean ecosystem services. *Science*, 314: 787-790.

CHAPTER 2

Assessing changes in ecosystem functioning and services via functional traits of an estuarine fish assemblage

Assessing temporal variation in ecosystem functioning and services via functional traits of an estuarine fish assemblage

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Abstract

Biodiversity affects ecosystem functioning and hence influences the delivery of ecosystem services. This study aimed to assess, via functional traits, the role of an estuarine fish assemblage in the provision of ecosystem services (links established based on literature) and investigate the possible temporal and spatial variations. Fish were sampled in the Mondego estuary throughout a decade (2003-2013) and characterized according to five traits (mobility, maximum body size, diet, feeding mode and salinity preference). Results suggest that the fish assemblage of the Mondego estuary has the potential to contribute to all regulating ecosystem services (except air purification), although not equally, as the seven functional groups of fish are linked with specific services, they were not equally represented in the assemblage and varied through space and time. Feeding mode, diet and body size were the most important functional traits to regulating services and their effects depended on both service and ecosystem. Trait variation was strong, spatially and inter-annually, with a weak seasonal effect. Despite the overall temporal and spatial variability in functional redundancy, results suggest that nutrient cycling, biological control, waste treatment and assimilation and climate regulation are likely the services to which the fish assemblage of Mondego estuary contributes in a more resilient manner. This study highlighted the importance of fish in the delivery of regulating ecosystem services in estuaries and the influence of environmental variables in fish functional traits, which needs to be taken into account, as well as the resilience of the ecosystem services provided, to establish conservation efforts.

Keywords: fish assemblage, estuaries, ecosystem functioning and services, functional traits, functional redundancy, resilience

Introduction

Ecosystems contribute greatly to human welfare, being strongly connected to human societies (Costanza *et al.* 1997; Kremen 2005; Luck *et al.* 2009; García-Llorente *et al.* 2011). The concept of ecosystem services – "the direct and indirect contributions of ecosystems to human well-being" (de Groot *et al.* 2010) – has become increasingly used because it highlights the value of ecosystems to humans, which is not fully perceived, despite its extreme importance (Boyd & Banzhaf 2007). Humans depend on

ecosystems for a variety of purposes, from the production of food and medicine to the provision of natural services such as cleansing, recycling and maintenance of a suitable climate, which are essential to human survival (Daily 1997; Kremen 2005). As such, ecosystem services are of vital importance and need to be taken into account in ecosystem conservation and management strategies (Costanza *et al.* 1997; Daily 1997). In addition to the intrinsic value of biodiversity (of taxonomic, functional and phylogenetic diversity) it is fundamental to human well-being (Díaz *et al.* 2011) because it affects ecosystem functioning - i.e. "ecological processes that control the fluxes of energy, nutrients and organic matter through the environment" (Cardinale *et al.* 2012) - and hence it influences the provision of ecosystem services such as biomass production, nutrient cycling and soil formation (Díaz *et al.* 2006; Cardinale *et al.* 2012; Harrison *et al.* 2014). Therefore, through ecosystem functioning, humans can obtain ecosystem services.

Several typologies of ecosystem services have been proposed (Millennium Ecosystem Assessment 2005; Beaumont *et al.* 2007; Fisher *et al.* 2009; Böhnke-Henrichs *et al.* 2013; Liquete *et al.* 2013). We selected the widely accepted classification from de Groot *et al.* (2010) for this study, according to which there are four types of ecosystem services: provisioning (e.g. food and raw materials), regulating (e.g. climate regulation and biological control), habitat (e.g. maintenance of life cycles of migratory species and gene pool protection) and cultural (e.g. aesthetic values and information for cognitive development). In the last two decades, there was a marked increase of investigation related to ecosystem services (Fisher *et al.* 2009; Liquete *et al.* 2013), although this field is relatively recent and needs more research, especially concerning services mediated by several biological groups such as fish. For the latter, existing information is scarce and needs updating. As such, it is urgent to gain knowledge and new perspectives of the role of fish in ecosystem functioning and ultimately in generating ecosystem services, which could be useful in management of fisheries and potential programs of biodiversity conservation. Furthermore, loss of biodiversity is closely associated with loss of ecosystem services (Worm *et al.* 2006). Thus, it is paramount to measure and estimate the effects of disturbance on biodiversity patterns to conduct conservation efforts and management of natural resources (Mouillot *et al.* 2012).

Human activities for subsistence have led to over-exploitation of natural resources and habitat loss and changes, leading to alterations in taxonomic and functional structure of biological assemblages (Mouillot *et al.* 2012; Henriques *et al.* 2013; Muntadas *et al.* 2016), and thus affecting ecosystem functioning and mostly limiting their capacity to provide essential services (Kremen 2005; Cardinale *et al.* 2012). For example, larger fish species are most affected by size-selective fisheries, which can have implications such as trophic cascades and a consequent decline of biological control (Genner *et al.* 2010). Furthermore, multiple trade-offs between human actions and ecosystem services may occur (Hattam *et al.* 2015), for example destruction of habitats by deforestation to obtain timber in turn leads to loss of other ecosystem services such as climate regulation through carbon sequestration by plant species (Cardinale *et al.* 2012; Mouillot *et al.* 2012). In fact, the increasing demand for provisioning services over the last century has caused a decrease in regulating services (Millennium Ecosystem Assessment 2005; Carpenter *et al.* 2009; Pinto *et al.* 2014), thus it is crucial to develop research about this type of services, which is essential to regulate environmental quality (TEEB 2010).

The identification of specific components of biodiversity providing a given ecosystem service is an important step in ecosystem services assessment (Luck *et al.* 2009; García-Llorente *et al.* 2011). Several studies have identified functional traits as good indicators of ecosystem processes and therefore, useful tools to ecosystem services assessment (Hooper *et al.* 2005; Díaz *et al.* 2007; de Bello *et al.* 2010). Functional traits are the characteristics of organisms that are measurable at the individual level and have

direct influence on organisms' performance (Mouillot *et al.* 2012) and as such, they influence organisms' fitness in the environment (response trait) and its effects on ecosystem processes or services (effect trait) (Violle *et al.* 2007; de Bello *et al.* 2010). And functional trait composition of an ecosystem can have a notable effect on its ecosystem functioning (de Bello *et al.* 2007; Díaz *et al.* 2007; Díaz *et al.* 2011; García-Llorente *et al.* 2011). Hence, linkages between biodiversity and ecosystem services become more explicit by describing biodiversity through the traits of organisms than through species number or abundance only (Díaz *et al.* 2007; de Bello *et al.* 2010; Laliberté *et al.* 2010; Díaz *et al.* 2011). Therefore, functional traits are strong candidates to quantify ecosystem service delivery (Hooper *et al.* 2005; Kremen 2005; de Bello *et al.* 2010; Díaz *et al.* 2011; Lavorel & Grigulis 2012). Most evidence of links between functional traits and ecosystem services has been reported for plant communities (de Bello *et al.* 2010). However, for animals, numerous connections between behavioral traits (e.g. feeding mode, mobility) and morphological traits (e.g. body size and mandibular structure) and the provision of ecosystem services have also been documented (Carpenter *et al.* 1985; Deegan 1993; Hall *et al.* 2007; de Bello *et al.* 2010). There is a need for further research about evidence for trait-service relationships, especially concerning fish.

Although functional trait composition of a community presents an undeniable connection to provision of ecosystem services, it is necessary to know which functional traits are represented by the species in a community, because it influences the ecosystem services being provided (Muntadas *et al.* 2016). For example, in coral reefs, biodiversity and functional complexity is generally high, but a major ecosystem process, such as bioerosion, may depend on the activity of a single group of species such as parrotfishes (i.e. grazer species that remove carbonate from the reef matrix while feeding on coral tissue), which makes this process more vulnerable to disturbance (Bellwood *et al.* 2003). Functional redundancy, i.e. different species sustaining similar functional roles, may insure the maintenance of ecosystem functioning in a situation of stress with subsequent degradation of biodiversity (Walker 1992; Fonseca & Ganade 2001; Bellwood *et al.* 2004; Mouillot *et al.* 2013; Mouillot *et al.* 2014). Functional redundancy has been related to ecosystem resilience – "the capacity of an ecosystem to absorb a given amount of disturbance without compromising its stability and to retain essentially its characteristics" (Holling 1973; Walker *et al.* 1999; Gunderson 2000; Folke *et al.* 2004). Nevertheless, if a set of species sustains similar functions but is equally affected by disturbance, functional redundancy does not ensure the resilience of those functions (Hughes *et al.* 2005; Muntadas *et al.* 2016). Moreover, it has been noted that rare species can have a significant effect in the maintenance of functions in disturbed ecosystems (Boero 1994; Walker *et al.* 1999; Lyons *et al.* 2005), because they may show different responses to stress and sustain ecosystem functioning under changing environmental conditions, where the abundance of common species with equivalent functions has decreased (Walker *et al.* 1999; Mouillot *et al.* 2013).

Estuarine and coastal systems are extremely productive and valuable (Costanza *et al.* 1997). For example, estuaries are important habitats for several fish, including commercial species that use them for shelter, feeding, nursery and migratory purposes (Elliott *et al.* 2007; Baptista *et al.* 2015a). Yet human-induced alterations in estuaries and environmental changes may have significant effects in the structure and composition of their fish assemblages (Vasconcelos *et al.* 2007; Baptista *et al.* 2010). Notably, alterations of environmental variables such as salinity, temperature and precipitation can lead to functional changes in estuarine fish assemblages (Smolders *et al.* 2000; Garcia *et al.* 2001; Martinho *et al.* 2007). In fact, estuaries are among the most exploited natural systems worldwide (Worm *et al.* 2006; Barbier *et al.* 2011) and their degradation is affecting their ecological integrity and the provision of critical ecosystem services, such as the number of viable fisheries, the provision of nursery habitats and natural cleansing

processes of water (Kennish 2002; Worm *et al.* 2006; Borja *et al.* 2010; Barbier *et al.* 2011; Dolbeth *et al.* 2011).

The main aim of this study was to assess the potential influence of an estuarine fish assemblage on the provision of ecosystem services exerted via functional traits, and possible temporal variations. The first objective of the study was to obtain information on links between fish functional traits and service provision based on a literature review. Moreover, we used the fish assemblage of the Mondego estuary (Northeast Atlantic) as a case study since it has been monitored for over 10-years. Furthermore, additional objectives were explored for this case study, namely: (1) to develop a functional characterization of the fish assemblage in order to identify fish functional groups within the estuary, and their possible contribution to the provision of regulating ecosystem services; (2) to investigate patterns of fish functional traits and functional groups and their underlying environmental drivers; and (3) to explore the resilience of ecosystem services provided by the fish assemblage through functional redundancy (sensu de Bello *et al.* 2007).

Materials and methods

Study area

The Mondego estuary is a mesotidal system influenced by a warm-temperate climate, located on the North East Atlantic - Portugal (40°08'N, 8°50'W) (Verdelhos *et al.* 2014; Alves *et al.* 2015). This estuary shows a typical longitudinal estuarine gradient, with depth, dissolved oxygen and salinity increasing from upstream to downstream and reaching the highest values near the mouth (Leitão *et al.* 2007). This small estuary of 8.6 km^2 (Verdelhos *et al.* 2014; Baptista *et al.* 2015b) has a length of 21 km (Alves *et al.* 2015) and comprises two arms, separated at 7 km from the shore by the Morraceira Island (Verdelhos *et al.* 2014), that join again near the mouth (Baptista *et al.* 2015b). The two arms present different hydrological characteristics: (1) the south arm is shallower (2-4 m during high tide), presenting about 75% of intertidal mudflats; (2) the north arm is deeper, 5-6 m at high tide (Baptista *et al.* 2013; Alves *et al.* 2015). The lower portion of the north arm is dredged frequently to maintain its depth, since it is the main navigation channel of the Figueira da Foz harbour (Baptista *et al.* 2015b).

The Mondego estuary has an important economical value, supporting industrial activities such as salt work, agricultural areas, mercantile and fishing harbours, which make it vulnerable to numerous physical impacts and high loadings of nutrients (Veríssimo *et al.* 2013). The estuary has suffered several anthropogenic pressures and hydromorphological transformations over the last decades (Neto *et al.* 2010; Veríssimo *et al.* 2013). From 1993 to 1997, there was an interruption of the connection between the two arms (Baptista *et al.* 2015b). As a result, the river flowed mainly through the north arm, and water circulation in the south arm was mostly dependent of tides as well as on the reduced freshwater input from the Pranto River (Verdelhos *et al.* 2014). In 1998, the communication between the two arms was re-established after a restoration plan (Baptista *et al.* 2015b), leading to a gradual ecosystem recovery (Verdelhos *et al.* 2014). In addition, several extreme climatic events have been observed in the last 15 years, including floods in 2000-2001 and droughts in 2004-2005 (Martinho *et al.* 2007; Baptista *et al.* 2010; Baptista *et al.* 2015b). These events affected some characteristics of the estuary, such as salinity and water temperature, and consequently changing the structure of the fish assemblage (Baptista *et al.* 2015b).

Within the Mondego estuary, the main types of estuarine habitats are intertidal soft substratum (composed of sediments such as silt and sand), salt marsh (macrophyte-dominated and saline-influenced habitats) and subtidal soft substratum (permanently subtidal unvegetated habitats, composed of sediments)

(França *et al.* 2009). The downstream areas of the south arm have important seagrass beds (Dolbeth *et al.* 2008). These seagrass habitats show higher diversity, productivity and functional organization of benthic organisms, as well as longer and more complex food webs, than the sandflat habitats (Cardoso *et al.* 2004; Verdelhos *et al.* 2005; Dolbeth *et al.* 2008; Dolbeth *et al.* 2015). In the Mondego estuary, primary production is mostly performed by phytoplankton (Rodrigues & Pardal 2015), with *Zostera noltii* leaves and *Gracilaria gracilis* contributing equally to the productivity of the estuary, followed by *Z. noltii* belowground and finally by *Ulva spp* (Rodrigues & Pardal 2015). In the fish assemblage, the most abundant species are the estuarine residents such as *Pomatoschistus microps* and *Pomatoschistus minutus*, species that use the estuary as nursery grounds such as *Dicentrarchus labrax*, *Solea solea* and *Platichthys flesus*, and marine migrant species like *Diplodus vulgaris* (Martinho *et al.* 2007). This estuary supports many fish species with high socio-economic importance (e.g., *Anguilla anguilla*, *Chelon labrosus*, *D. labrax*, *D. vulgaris*, *Liza ramada*, *P. flesus*, *S. solea*) (Vasconcelos *et al.* 2009; Baptista *et al.* 2013). Several studies provided evidence of changes in this fish assemblage due to anthropogenic impacts. For instance, Leitão *et al*. (2007) observed a lower taxonomic diversity of fish species in 2003-2004 than in 1988-1992, which may be a result of dredging activities and bank reclamation in the north arm and eutrophication in the south arm. More recently, other changes in fish assemblage composition, structure and production were related to several hydrology changes due to shifts in precipitation and river flow (which affect salinity) and temperature (Dolbeth *et al.* 2008; Martinho *et al.* 2010; Baptista *et al.* 2015b).

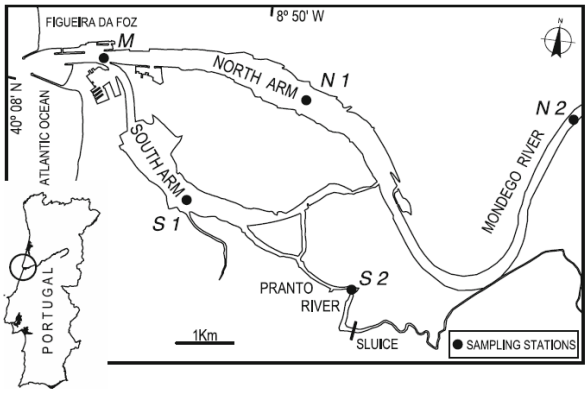


Figure 2.1 - The Mondego estuary, with the location of the five sampling stations.

Sampling procedures

The fish assemblage of the Mondego estuary was sampled monthly, during high water of spring tides, from June 2003 to November 2013 (every two months between January 2007 and March 2010). There were no sampling campaigns in September and October 2004 and in May, July, October, November and December 2008, due to technical constraints or bad weather.

Fish surveys were carried out at five stations (M, S1, S2, N1, N2; Figure 2.1), following a salinity gradient, starting downstream with station M showing higher salinity due to its proximity to the estuary mouth and stations S and N presenting lower salinities, since they are located more upstream. Stations N1 and N2 (lowest salinity), in the north arm, are deep and characterized by high runoff from the Mondego river. Stations S1 and S2, located in the south arm, are shallower and their runoff depends on inputs from the Pranto river, a small tributary of the Mondego river.

Fishing was performed during the night, using a 2-m beam trawl with one tickler chain and 5-mm stretched mesh size in the cod end. At each station, three replicate trawls were made, each for an average of 3 min, covering a minimum area of 500 m^2. After the collection, fish were kept on ice, transported to the laboratory and preserved frozen until processing, when they were identified, counted, measured (total length to nearest 1 mm) and weighted (wet weight to the nearest 0.01 g).

Environmental variables of stations were determined before each tow, namely: water temperature (°C), salinity and dissolved oxygen (%), all measured from bottom water. Monthly precipitation values (mm) were acquired for the Soure 13F/01G monitoring station, from INAG – Portuguese Water Institute. Monthly freshwater runoff (dam^3) was obtained from INAG station Açude Ponte Coimbra, 12G/01A, near the city of Coimbra (located 40 km upstream). North Atlantic Oscillation index (NAO) was obtained from the National Oceanic and Atmospheric Administration (NOAA) webstation (http://www.noaa.gov/).

Functional characterization of the fish assemblage

To characterize the fish assemblage of the Mondego estuary, we first classified each species captured in the study period according to its taxonomy, namely: class, order and family. Second, we classified each species according to its functional traits: mobility (adapted from Henriques *et al.* 2013), maximum body size (adapted from Baptista *et al.* 2015b), diet (adapted from Elliott *et al.* 2007), feeding mode (adapted from Costello *et al.* 2015) and salinity preference (adapted from Whitfield *et al.* 2012). This set of traits has been previously used in other studies concerning functional diversity (Elliott *et al.* 2007; Nyitrai *et al.* 2012; Baptista *et al.* 2015b), although salinity preference is not considered a trait in itself, according to Verberk *et al.* (2013), since it rather reflects an interaction of a trait with environmental conditions. All traits were considered as qualitative, except salinity preference, in which the categories were considered as binary (Table 2.1). To characterize the functional traits related with habitat use and ecology (i.e. diet and feeding mode) we took into account the life stages and ecology of each fish species within the estuary. Information was collected from published literature and publicly available databases such as FishBase (Froese and Pauly 2015), Marine Species Identification Portal and International Union for Conservation of Nature (IUCN).

Table 2.1 - List of the fish functional traits compiled for all species.

Trait	Trait definition	Trait categories	Trait category description
Mobility	The ability of fish to move, which reflects the capacity of fish to respond to local changes in habitat and also the capacity to catch fish prey	High	Movement over large distances
		Medium	Daily movement of tens of meters
		Sedentary	Limited movement and well defined home ranges
		Territorial	Limited movement and territorial behavior
Body size	Considered as maximum body length, related to other life-history traits, energy and impacts on the food web	Large	> 40 cm
		Medium	26 – 40 cm
		Small	10 – 25 cm
		Very small	< 10 cm
Diet	Food preference, reflects trophic structure, distribution of resources and how fishes may adapt to the habitat	Planktivorous	Feeding mainly on plankton
		Invertebrate feeder	Feeding on small invertebrates
		Omnivorous	Feeding on algae, macrophytes, periphyton, epifauna and infauna
		Macrocarnivorous	Feeding on macroinvertebrates and vertebrates (mostly fish)
		Detritivorous	Feeding on detritus or microphytobenthos
Feeding mode	Primary feeding method used by fish	Browser	Browsing on substrate, selecting plankton feeding
		Filterer	Filtering plankton
		Hunter	Hunting macrofauna (predator)
Salinity preference	Range of salinity tolerated by the fish species, reflects the ability to deal with osmotic stress	Marine (0 = no; 1 = yes)	Preference for salinities > 30
		Brackish (0 = no; 1 = yes)	Tolerance for a wide range of salinities (5 – 30)
		Freshwater (0 = no; 1 = yes)	Preference for salinities between 0 and 0.5

Relationships between fish functional traits and ecosystem services

To investigate the link between fish functional traits and the provision of ecosystem services we made an extensive literature review focusing on regulating ecosystem services, namely: nutrient cycling, coastal protection and disturbance prevention, maintenance of sediment processes and sediment redistribution, biological control, climate regulation, air purification, waste treatment and assimilation and regulation of linkages between ecosystems (Holmlund & Hammer 1999; Hattam *et al.* 2015; see definitions in Table 2.2). The direction of effects of traits on each ecosystem service, positive or negative, was also registered as found in literature, *sensu* de Bello et al. (2010). This methodology was also used by García-Llorente *et al.* (2011), who identified relevant ecosystem services and then worked their way backward to ecosystem properties and aquatic plant functional traits that supported them.

The research was conducted with ISI Web of Science and Google Scholar, using a list of keywords which always included the terms "fish AND (service* OR function*)" as well as one of the above referred ecosystem services. Previous classifications of these ecosystem services were also taken into account (Holmlund & Hammer 1999; Beaumont *et al.* 2007; de Bello *et al.* 2010; Böhnke-Henrichs *et al.* 2013; Liquete *et al.* 2013; Hattam *et al.* 2015). Relevant articles were compiled to extract data.

Data analysis

A hierarchical cluster analysis was used to identify functional groups within the Mondego estuary fish assemblage, based on their functional traits (matrix of 43 species x 5 traits). We used Ward's agglomerative method (Ward 1963) and Gower distance, also known as Gower's coefficient (1971), which is expressed as a dissimilarity that can be calculated between different types of variables, applying a standardization to each variable (Maechler *et al.* 2015; R Development Core Team, 2015).

A Principal Coordinates Analysis (PCO) was also conducted to investigate the similarity of species traits within the fish assemblage (Roberts 2016; R Development Core Team, 2016). This ordination technique applies a projection of the points into axes minimizing residual variation in the space of the dissimilarity measure chosen (Anderson *et al.* 2008). It is similar to Principal Components Analysis (PCA) but it allows the use of a broader range of distances and dissimilarity coefficients being better suited to ecological datasets, as proposed by Gower (1966).

Fish trait categories were quantified in three ways, with: the density, biomass and number of species representing each trait category in a sample, where each sample is the mean assemblage sampled in a given station and date.

To statistically test spatial and temporal differences of each of the functional groups previously identified in the cluster analysis, we used multivariate analysis of variance using permutations (PERMANOVA) in PRIMER 6 package with PERMANOVA + (Clarke & Gorley 2006; Anderson *et al.* 2008). To this end we considered three fixed factors: season (autumn, winter, spring, summer), station (M, N1, N2, S1, S2) and year (from 2003 to 2013). PERMANOVA uses permutation methods for testing the simultaneous response of one or more variables to one or more factors in an analysis of variance experimental design on the basis of a resemblance measure (Anderson *et al.* 2008). It assumes only that the samples are independent and identically distributed under a true null hypothesis. Post-hoc pair-wise tests were performed when main tests were significant.

To investigate the relationship of each functional trait in the assemblage with environmental variables (measured in density, biomass and number of species representing each trait category in each sample) we performed generalized linear models (GLM) (R Development Core Team, 2005). For each trait category, two GLM were produced: one considering only the continuous environmental variables (salinity, temperature, dissolved oxygen, precipitation and NAO) and another also considering the factors season, station and year. Several plots were then used to help visualize the effects of significant variables and factors on each trait as fitted in the GLM (Breheny & Burchett 2016; R Development Core Team, 2016). Hierarchical Partition of Variance was also performed to assess the relative importance of each variable to trait variation (Nally 1996; Grömping 2006; R Development Core Team, 2006).

In all the above-mentioned analyses, density and biomass data were square root transformed to reduce right skewness and the effect of outliers (Anderson *et al.* 2008). Resemblance matrices for biomass and density were based on Bray-Curtis dissimilarity while for the species number the Euclidean distance was used.

Finally, to assess the resilience of the ecosystem services supported by the Mondego estuary's fish assemblage, the functional redundancy of each sample was calculated based on trait density and biomass data. Functional redundancy (FR) was determined as the difference between Gini-Simpson index of species diversity (SD) and functional diversity (FD) of the sample considered as Rao's quadratic entropy based on their functional dissimilarity (de Bello *et al.* 2007; Debastiani & Pillar 2012; R Development Core Team, 2015), expressed as:

$$FR = SD - FD \quad (2.1)$$

Functional redundancy is zero if all species are functionally different and equal to Gini-Simpson index if all species are functionally identical (de Bello *et al.* 2007).

Results

Literature review of trait – ecosystem service relationships

The literature review allowed identifying connections between fish functional traits and regulating ecosystem services, namely reported as a mechanistic link or a concomitant measurable change in a given functional trait category in an assemblage and a given ecosystem service (qualitative relationships in Figure 2.2 and Table 2.2).

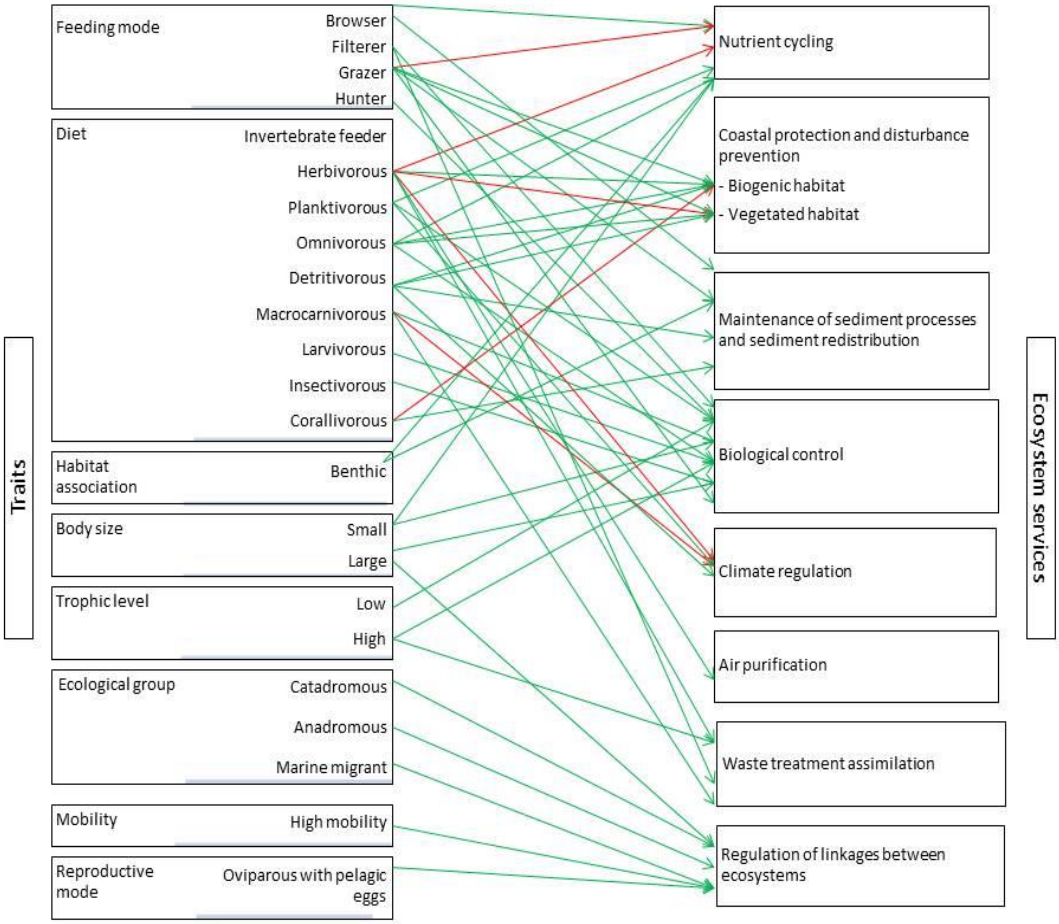


Figure 2.2 - Illustration of fish traits and ecosystem services general relationships. Green arrows indicate positive relationships and red arrows indicate negative relationships between each trait and ecosystem service.

The two traits with more evidence of links with ecosystem services were diet and feeding mode. The regulating ecosystem services with more connections to fish traits were nutrient cycling, coastal protection and disturbance prevention, biological control and regulation of linkages between ecosystems. It is important to refer that four of the traits represented in Figure 2.2 and Table 2.2 were not analyzed in our case study of the Mondego estuary, namely: habitat association, trophic level, ecological group and reproductive mode. Actually, from these, only the latter is considered a trait, according to Costello *et al.*

(2015), with the other ones being the result of a combination of traits (Elliott *et al.* 2007). Also, some trait categories from feeding mode (grazer) and diet (herbivorous, larvivorous, insectivorous and corallivorous) were not considered in the analysis of our case study of the Mondego estuary because they were not present in the fish assemblage. These trait categories were only considered for the literature review, which was conducted for general relationships between fish traits and ecosystem services, in all aquatic habitats.

Some single trait categories showed different effects on ecosystem services depending on the context/ecosystem. For instance, herbivorous fish could have a positive effect on protection of the shoreline by controlling macroalgal blooms in coral reefs (Nyström *et al.* 2000; Hoey & Bellwood 2011; D'Agata *et al.* 2014; Pratchett *et al.* 2014), but could have a negative effect on the same service by consuming large amounts of seagrass in vegetation habitats in lower latitudes, therefore reducing these natural barriers (Heck & Valentine 2006).

Table 2.2 – Summary of fish traits and ecosystem services relationships. Response trend is given within parentheses (+ for positive or – for negative). The classification of ecosystem services was adapted from Holmlund and Hammer (1999), de Groot *et al.* (2010) and Hattam *et al.* (2015).

Ecosystem service	Fish traits with reported effect on ecosystem service	Trait categories and response trend	Examples
Nutrient cycling: Natural cycling processes that allow the presence of nutrients in the water, for example: decomposition and mineralization, nutrient mobilization and availability, biogeochemical activity and biological productivity	Feeding mode, diet, habitat association, body size	Browser (+), grazer (-), herbivorous (-), planktivorous (+), omnivorous (+); benthic (+); small body size (+)	Planktivorous and benthivorous fish (like the common carp) have an important influence on nutrient regeneration and concentration, either by browsing feeding activity (Qin & Threlkeld 1990) or by digestive activity (Andersson *et al.* 1978). Grazer fishes may modulate the nature of nutrient cycling by primary producers, limiting significantly N concentrations on substrates they feed on, compared to substrates inaccessible to macroherbivores, as observed by Flecker *et al.* (2002). The gizzard shad (*Dorosoma cepedianum*) is an omnivorous fish that can be important to nutrient cycling, translocating nutrients from sediments to water and thus providing a subsidy of "new" nutrients into the water column to phytoplankton (Vanni *et al.* 2008). Because of the inverse relationship between body size and metabolic rates, small fish have the potential to excrete at higher rates than larger fish. In one case, a fish assemblage dominated by small-bodied planktivores recycled more P than an assemblage of large-bodied piscivores of equivalent biomass (Carpenter *et al.* 1992).
Coastal protection and disturbance prevention: Contribution of ecosystem structures to protect the coastal zone from erosion and attenuate the intensity of environmental disturbances (such as storm floods, tsunamis and hurricanes), through the production and maintenance of biogenic living structure (salt marshes, mangroves, seagrass beds, coral reefs)	Diet, feeding mode	Biogenic habitats: herbivorous (+), omnivorous (+), detritivorous (+), corallivorous (-), grazer(+); Vegetated habitats: omnivorous (+), detritivorous (+), herbivorous (-), small grazer (+)	Biogenic habitat: Herbivorous reef fishes (primarily grazing species) support a large range of critical functional roles and services, such as the control of macroalgal establishment, removal and transport of sediments to provide a hard and clean substratum for coral recruitment and the bioerosion of dead corals which facilitates resilience to disturbances such as bleaching events (Nyström *et al.* 2000; Burkepile & Hay 2008; Hoey & Bellwood 2011; D'Agata *et al.* 2014; Pratchett *et al.* 2014). Detritivorous fishes are also involved in reef resilience (Cheal *et al.* 2010). Omnivorous fishes such as *Diplodus* spp. are also characterized by high species diversity and a high degree of specialization (browser, grazer, scraper, crusher), having the greatest impact on the structure and dynamics of coral reefs, where they directly control algal communities (Ruitton *et al.* 2000). Corallivorous fishes directly affect the coral condition by mechanical damage and tissue removal, which also facilitates algal competition and spreading of disease pathogens (Rotjan & Lewis 2008; Raymundo *et al.* 2009). Chronic predation by corallivores may also exacerbate effects of coral disturbance (e.g. climate-induced coral bleaching), impeding reef recovery and causing further coral loss (Cole *et al.* 2008). Vegetated habitat: Small grazers (mesograzers), as well as the omnivorous pinfish, have a dominant role in controlling the biomass of algae growing epiphytically on seagrass leaves (Heck *et al.* 2000; Heck & Valentine 2006). Studies over the last two decades have shown that a number of herbivorous fish species in lower latitudes ingest large amounts of aboveground seagrass biomass (Heck & Valentine 2006).
Maintenance of sediment processes and sediment redistribution: Processes involved in the structuring of bottom conditions and availability of sediments, such as soil formation, sedimentation, bioturbation and activity of benthic organisms	Habitat association, feeding mode, diet	Benthic (+), browser (+), grazer (+), detritivorous (+),	Fish activity induces mixing and resuspension of the soil (Avnimelech *et al.* 1999). Gelwick *et al.* (1997) describe how benthic algivorous fishes resuspend silt, detritus, and other particulate organic matter from the bottom into the current, as previously described by Flecker (1992) for a diverse fauna of grazing fishes (Holmlund & Hammer 1999). Bioturbation, accomplished by benthivorous fish, alters the structure and properties of the sediment and thereby influences diffusive and/or advective transport of both solutes and particulate matters. It also affects the underlying sediment layers (Adámek & Maršálek 2013). Adult carp (*Cyprinus carpio*) forage predominantly on benthic macroinvertebrates and, when doing so, they induce severe bioturbation of the pond bottom (Adámek & Maršálek 2013). Katz *et al.* (2002) concluded that while foraging for food and swimming near the sediment surface, mullets resuspend detritus, increase the oxygen supply to the benthos and oxygenate buried organic matter, thereby dramatically improving the status of organically enriched, reduced sediments within a relatively short period of time.

Table 2.2 (continued).

Ecosystem service	Fish traits with reported effect on ecosystem service	Trait categories and response trend	Examples
Biological control: Maintenance of natural healthy population dynamics, regulation of food web dynamics and control of invasive species, pest regulation, storage and transfer of minerals and energy in food chains, maintenance of resilient and robust community structure	Body size, diet, feeding mode, trophic level	Bottom-up control: low TL(+), planktivorous (+). Wasp-waist control: small planktivorous fish (+). Top-down control: High TL (+), large fish (+), macrocarnivorous (+), hunter (+). Control of diseases: Larvivorous (+), insectivores (feed on larvae/eggs of insects) (+). Control of invasions: Omnivorous (+), macrocarnivorous (+). Control of algal blooms: grazer(+), filterer(+)	Bottom-up control: The peak landings of piscivores (high trophic level) later in the time series than for zooplanktivores (low trophic level), appears consistent with an overall bottom-up response to the earlier increase in abundance of forage fish (Caddy & Garibaldi 2000). Wasp-waist control: In eastern boundary current systems such as the California Current and the Humboldt Current, zooplankton biomass has been observed to have decreased over the past few decades. These long-term declines in zooplankton may also be related to major changes in the abundance of small pelagic fishes(Verheye & Richardson 1998). Top-down control: The strongest evidence for a key role of predators in controlling subtidal reef communities in the southern hemisphere is from two New Zealand marine reserves where there has been a decline in urchin densities and an associated change from urchin barrens to kelp over a 20-year period (Shears & Babcock 2002) because of the density and size of urchin predators (two of them are fish species). A rise in piscivore biomass brings decreased planktivore biomass, increased herbivore biomass, and decreased phytoplankton biomass (Carpenter *et al.* 1985). Large fish species eat the smaller ones, being between one or three orders of magnitude larger than their prey in terms of body mass (Strange *et al.* 1999; Woodward *et al.* 2005). Control of diseases: Larvivorous fishes (*Gambusia holbrooki, Aphaniusdispar dispar* and *Aphanius* sp.) are used as biological agents in malaria control (Shahi *et al.* 2015). *Gambusia affinis* can be an effective biological control agent in rice fields, feeding on mosquitoes (*Culex tarsalis* and *Anopheles freeborni*) and thus preventing diseases such as arboviruses and malaria (Hoy *et al.* 1972). Control of invasions: There are fish species that control invasive fish species, either on their adult phase, having a macrocarnivorous diet - for example the Caribbean grouper is a natural biocontrol of the invasive lionfish in coral reefs (Mumby *et al.* 2011)- or on initial phases of development, having an omnivorous diet - for example the Japanese dace consumes smallmouth bass eggs, an exotic species in Japan which is a threat to native biodiversity (Iguchi & Yodo 2004). Control of algal blooms: Removal of grazers from shallow-water environments may prompt increases in macroalgal standing crops that could be considered to be blooms (Valiela *et al.* 1997). Silver and bighead carp, both filter-feeding, are highly effective in controlling noxious blooms of algae (Xie & Liu 2001).
Climate regulation: Contribution of the biotic elements of an ecosystem to the conservation of a favourable climate through hydrological processes, regulation of climate-influencing substances, biogeochemical processes and heat exchange	Diet	Macrocarnivorous (-), herbivorous (-), planktivorous (+), detritivorous (+)	Experiments comparing two nutrient-enriched lakes in USA have linked the composition of fish communities with ecosystem carbon fixation. One of the lakes, with zooplanktivorous fish became a carbon sink (because zooplankton were suppressed, and primary producers - carbon fixers - were released from grazing pressure) and the other lake, with piscivores, became a carbon source (because the piscivores supressed the abundance of zooplanktivores, allowing the zooplankton community to exert a high grazing pressure on phytoplankton), thus indirectly mediating the flux of carbon between the lake and the atmosphere (Schindler *et al.* 1997). Herbivorous fishes have a negative influence on carbon fixation because they feed on primary producers (Holmlund & Hammer 1999). The flannelmouth characin (*Prochilodus mariae),* a detritivorous fish, regulates fundamental ecosystem processes, synthesis and degradation of organic carbon. Removing *Prochilodus* altered multiple components of organic carbon flow. This fish changed the composition of microbial biofilms to attached nitrogen-fixing cyanobacteria. By removing particles that reduce light essential for N fixers, they may facilitate a source of primary production that is independent of N limitation (Taylor *et al.* 2006).

Table 2.2 (continued).

Ecosystem service	Fish traits with reported effect on ecosystem service	Trait categories and response trend	Examples
Air purification: Regulation of the concentration of air pollutants in the atmosphere by the ecosystem components through absorption	Diet	Herbivorous (+)	The herbivorous damselfishes enhance several reef processes such as primary production (Hixon & Brostoff 1996), recovery of reef corals (Done *et al.* 1991) and nitrogen fixation since cyanobacteria are more common within their territories than outside (Hixon & Brostoff 1996). Damselfish territoriality influences the dynamics of some reefs by increasing the biomass of the algal turf and thus increasing reef productivity, since blue-green algae, potential nitrogen fixers, occur in these algal turfs (Brawley & Adey 1977).
Waste treatment and assimilation: Removal of contaminants and organic nutrients added to the environment by humans through ecosystem processes, such as: degradation, mineralization, transformation and conversion of toxicants to less toxic substances; burial of toxicants through bioturbation; sequestration of toxicants by living organisms; dilution and dispersal of toxicants through hydrodynamics; oxygen regulation in water	Trophic level, feeding mode, diet	High trophic level (+); filterer (+); herbivorous (+), macrocarnivorous (+)	Yellowfin tuna can record high mercury concentrations in their tissues due to biomagnification of this metal in the trophic chain and to the high position tuna occupy in the trophic chain (Ordiano-Flores *et al.* 2011). Silver and big-head carp, both filter-feeding, can accumulate PCBs (Zhou *et al.* 1999). One of the purification processes of Lake Taihu is nutrient feeding by fish (by eating aquatic plants), which accounts for 2039 to 2234 ton nitrogen and 140 to 150 ton phosphorus removal in water body per year (Han *et al.* 2015). The Baltic Sea fish community is a large sink for PCBs, with the most abundant species being sprat, herring and cod. Adult cods are predators of herring and sprat, and their livers have a much higher concentration of lipids and thus PCBs (which are highly lipophilic) (Mackenzie *et al.* 2004).
Regulation of linkages between ecosystems: Transportation and distribution of energy, nutrients and genetic material within and between different aquatic ecosystems (lakes, rivers, oceans, estuaries), ensuring the maintenance of migratory and nursery habitats	Ecological group, body size, mobility, reproductive mode	Catadromous (+), anadromous (+), marine-estuarine dependent(+) and marine-estuarine opportunist (+), large body size (+); high mobility (+); oviparous with pelagic eggs(+)	Several species of anadromous salmonid fishes migrate from marine environments where they spend most of their lives, to natal rivers to spawn and then die, and thereby transfer nutrients and carbon (Bilby *et al.* 1996). Catadromous European eels (*Anguilla anguilla*) in the Baltic Sea are one example of long-distant migrating fish species that transport nutrients, carbon and other substances from one part of the world's seas to another, spending most of their life in fresh or brackish waters where they feed and grow before they return to their spawning grounds in the Sargasso Sea, over 8000 km away, where they reproduce and then die (Holmlund & Hammer 1999). Connectivity between estuaries and adjacent coastal ocean is very important for a great number of marine migrant fish species, like *Solea solea* and *Diplodus vulgaris*, which use these sheltered estuarine areas as juveniles (Koutsikopoulos *et al.* 1989; Cabral & Costa 1999; Vasconcelos *et al.* 2008; Abecasis *et al.* 2009). These fish species spawn at sea where their pelagic eggs hatch, and ocean currents carry the larvae into estuaries, where they develop into juveniles and then move offshore after attaining a large proportion of their adult size (Deegan 1993). Home range or territory size tends to increase with body size (Woodward *et al.* 2005).

Case study – Mondego estuary

The environmental variables in the Mondego estuary showed seasonal, spatial and temporal variation (Figures 2.3-2.6). Salinity was higher in stations M and S1 and lower in station N2. Generally, lower salinities were registered in winter and higher salinities were registered in summer, with the highest salinity in 2007. Temperature was higher in station S2 and lower in station M, where oxygen values were generally higher. Oxygen was lower in station S2. The highest precipitation level was registered in autumn of 2006 and the highest runoff value was in winter of 2010. Temperature was higher in summer and lower in winter, and oxygen was generally lower in summer and higher in spring and winter.

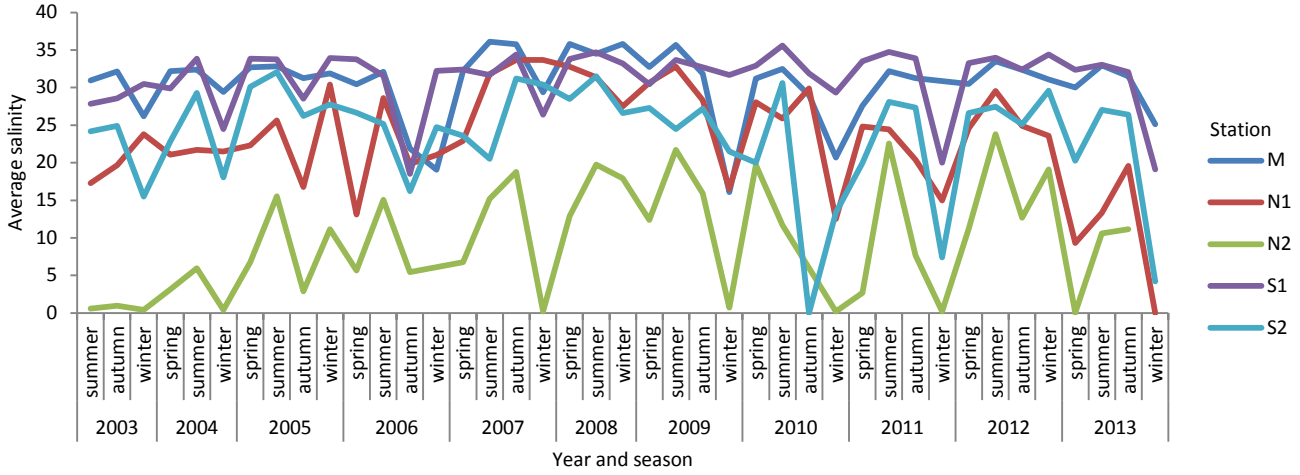


Figure 2.3 – Variation of salinity in the Mondego estuary.

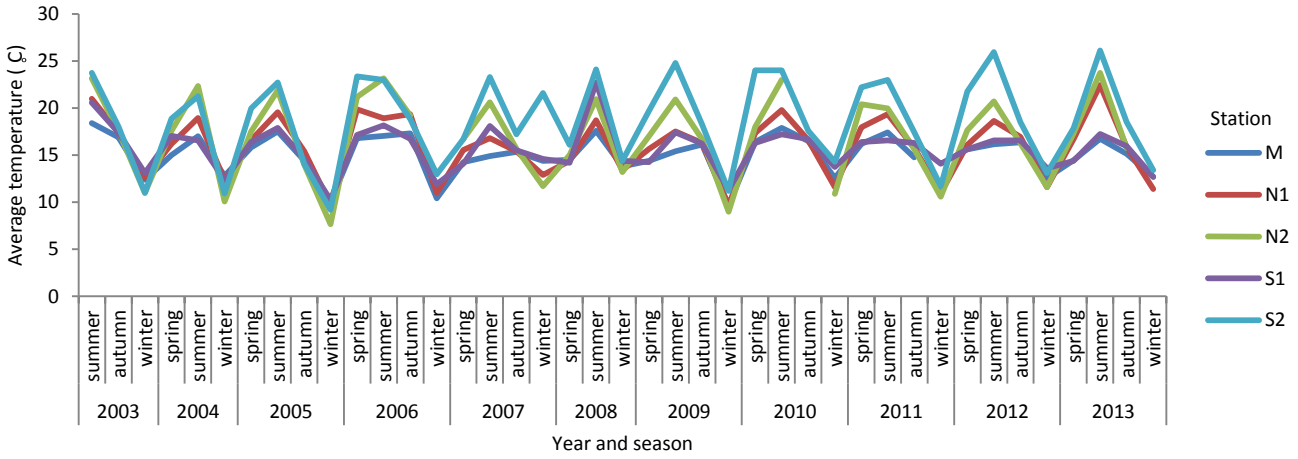


Figure 2.4 – Variation of temperature in the Mondego estuary.

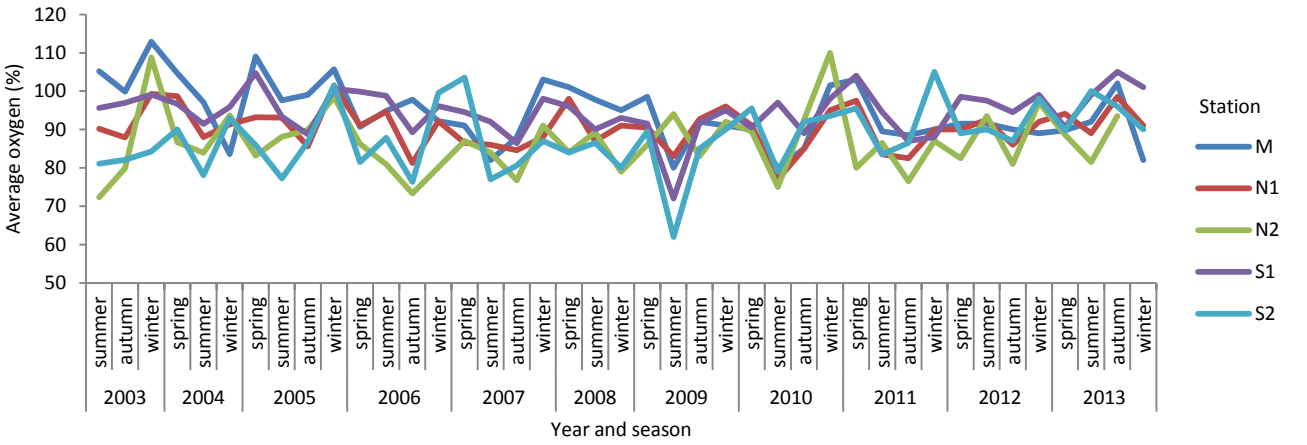


Figure 2.5 – Variation of oxygen in the Mondego estuary.

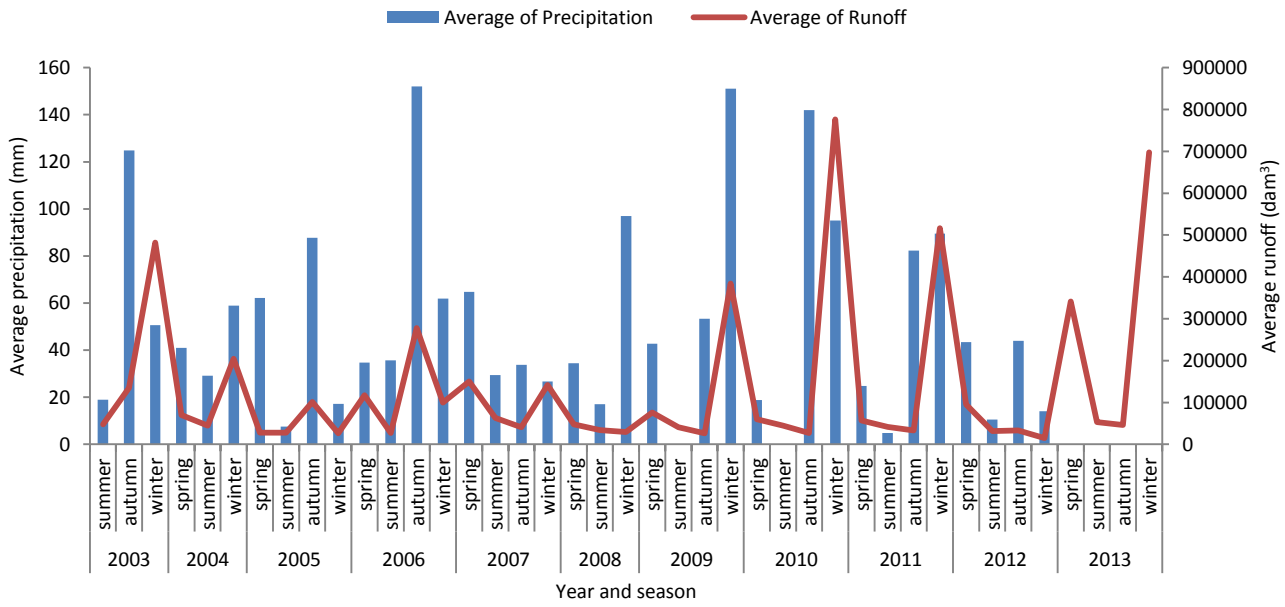


Figure 2.6 – Variation of precipitation and runoff in the Mondego estuary.

The hierarchical cluster analysis identified seven functional groups (FG) in the Mondego estuary fish assemblage which differ in their traits (Figure 2.7; Tables 1-3 - Appendix I). These FG may be able to provide different ecosystem services, as follows:

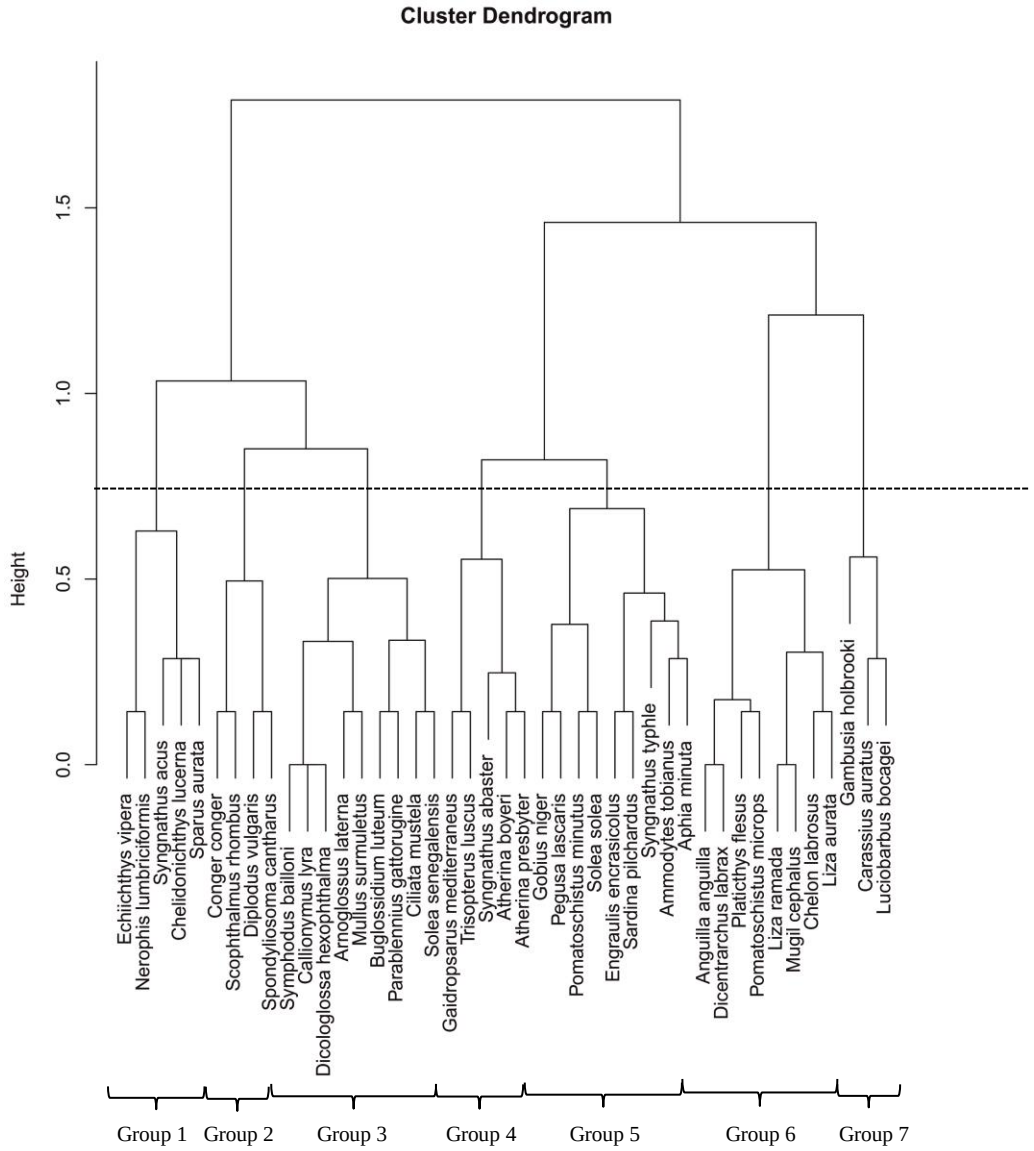


Figure 2.7 – Dendrogram produced by cluster analysis of the fish assemblage of the Mondego estuary based on five traits (mobility, body size, diet, feeding mode and salinity preference).

Group 1 – Fish species that feed by hunting other organisms. In general, fishes of this group are macrocarnivorous, prefer marine environments and are sedentary. Their feeding mode and diet make them very important for biological control, specifically top-down control and control of invasive species (Table 2.2). Two species have very small body size, which could mean they have an important role in nutrient cycling, because their excretion rates are higher than larger species (Table 2.2). However, the potential role of these very small fish species in the delivery of this ecosystem service depends on their abundance.

Group 2 – Marine fish species with high mobility, with an important role in linking the estuary and other ecosystems, reinforced by the fact that half of the species have large body size (Table 2.2). There are browsers in this group, which may mean they influence provision of services like nutrient cycling, maintenance of sediment processes and sediment redistribution (Table 2.2).

Group 3 – Invertebrate feeders with a salinity preference for marine environments. Most have small body size, which means they possibly contribute to nutrient cycling, and browsing feeding mode, which can be indicative of an important role in the maintenance of sediment processes and sediment redistribution (Table 2.2). Two species have high mobility, which could contribute to the regulation of linkages between the estuary and other aquatic systems, although that would depend on the abundance of these species.

Group 4 – Hunter fish species with small body size, also invertebrate feeders and omnivorous fishes. These fish species may have a substantial influence in nutrient cycling due to small size and omnivorous diet, although this depends on their abundance. This type of diet can also contribute indirectly to the provision of services like coastal protection and disturbance prevention, by controlling the growth of epiphytes in seagrass beds and therefore protecting these structures (Table 2.2). Also, omnivorous diet contributes to biological control, which is also provided by the hunters in the group. All members are marine and brackish species, although two can also tolerate freshwater.

Group 5 – Heterogeneous in terms of functional traits, although most members have small body size - a characteristic usually connected to nutrient cycling. Includes filter feeding species, which could provide ecosystem services like biological control and waste treatment and assimilation, and all planktivorous species, which may be linked to services such as nutrient cycling, biological control and climate regulation (Table 2.2).

Group 6 – Browser fishes with various diets, most with medium body size. All members tolerate all types of salinities (marine, brackish and freshwater) and most have high mobility, which suggests they can live in different aquatic environments and migrate between them, potentially contributing for the regulation of linkages between ecosystems. Their feeding mode and wide diet possibilities can also be indicators of their role in nutrient cycling, maintenance of sediment processes and redistribution, biological control (specifically, control of invasions), coastal protection and disturbance prevention, the latter service provided by omnivorous fish who protect biogenic structures by controlling algal communities (Table 2.2). Two species have a detritivorous diet, which could contribute indirectly to climate regulation, because they may remove particles that reduce light for primary producers, therefore facilitating carbon fixation (Table 2.2).

Group 7 – Freshwater omnivorous species, which can contribute to nutrient cycling, coastal protection and disturbance prevention and biological control (Table 2.2). Two browser species possibly contribute to maintain sediment processes and redistribution.

Functional groups 5 and 6 were the most represented in the Mondego estuary fish assemblage (in terms of biomass, density and number of species; see Figure 2.8-2.10). Group 7 had lower number of species and density, while group 4 showed the lowest biomass.

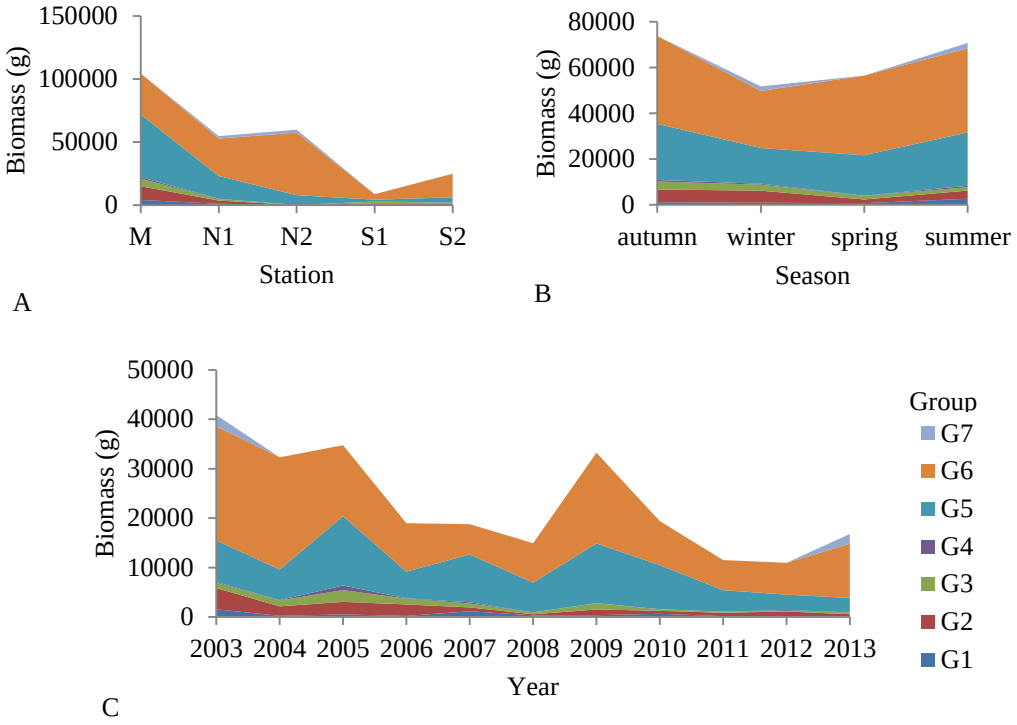


Figure 2.8 – Variation of fish functional groups (quantified by the sum of cumulative biomass of a group in all samples) in the Mondego estuary. A – Spatial variation. B – Seasonal variation. C – Temporal variation.

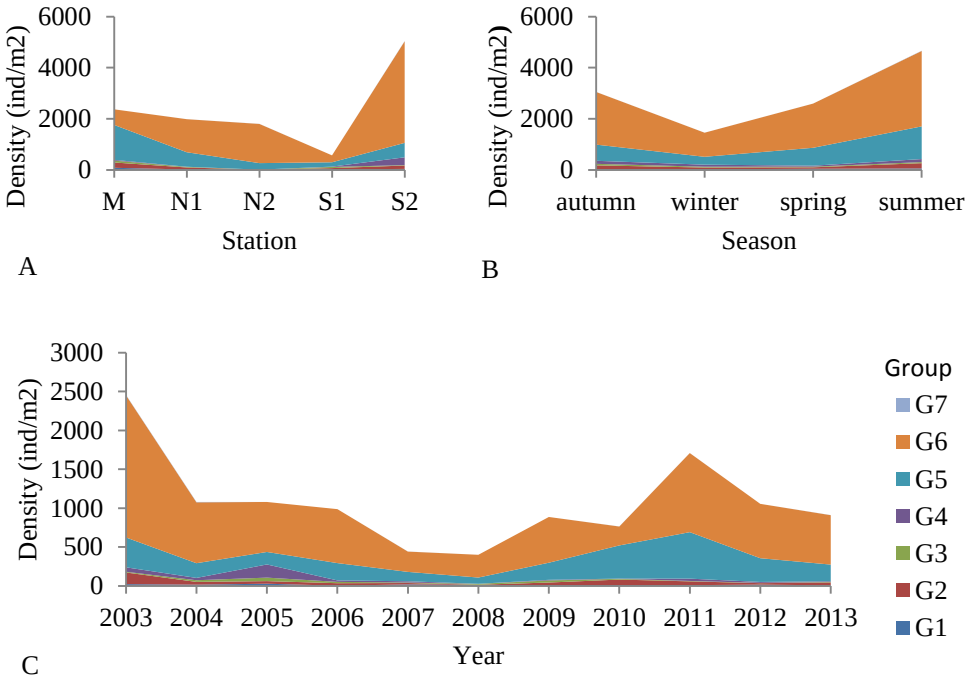


Figure 2.9 – Variation of fish functional groups (quantified by the sum of cumulative density of a group in all samples) in the Mondego estuary. A – Spatial variation. B – Seasonal variation. C – Temporal variation.

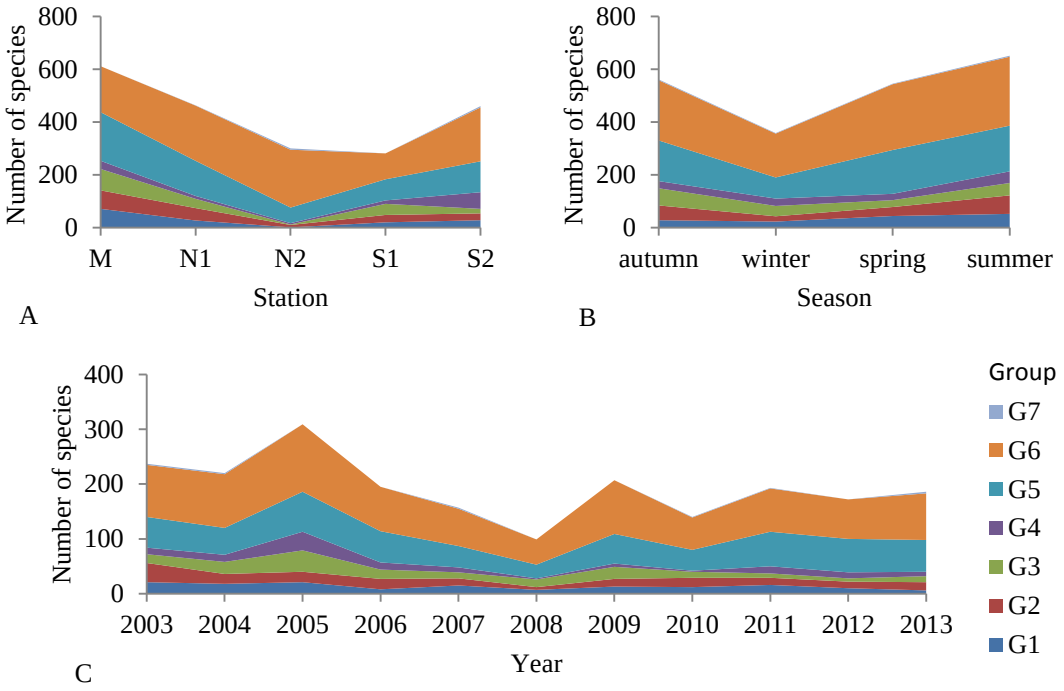


Figure 2.10 – Variation of fish functional groups (quantified by the sum of the cumulative number of species of a group in all samples) in the Mondego estuary. A – Spatial variation. B – Seasonal variation. C – Temporal variation.

Traits biomass, density and also number of species varied among functional groups, with group 6 presenting the highest percentage for high mobility and group 7 being the only group without marine species (Tables 1-3 - Appendix I). In terms of diet, invertebrate feeders were the most represented within the fish assemblage, especially concerning groups 3, 5 and 6. Omnivorous and macrocarnivorous were also well represented in the fish assemblage. Planktivorous were only present in group 5 and detritivorous were only present in group 6. The majority of the fish assemblage (by number of species) is characterized by high mobility (49%), small body size (42%), browsing feeding mode (60%), and salinity preference for marine water (51%; see Table 3 - Appendix I).

The ordination of fish species in the Mondego estuary considering their functional traits using Principal Coordinates Analysis (PCO) revealed patterns that complement and are in agreement with the information provided by the cluster analysis of traits alone (Figure 2.11). There is a clear separation between freshwater species and remaining species, although *Gambusia holbrooki* is closer to the rest of species because of its brackish salinity preference. Species from groups 4 and 5 are closer together in the PCO, probably because of their small body size and marine and brackish salinity preference. Group 2 shows the highest dispersion of species, with *Spondyliosoma cantharus* and *Diplodus vulgaris* being closer to other omnivorous species and *Conger conger* and *Scophthalmus rhombus* closer to other macrocarnivorous species. These four species also differ in their feeding modes, which could explain the differences in distribution in the plot. Two species of group 3, *Arnoglossus laterna* and *Mullus surmuletus*, are farther from the remaining species in their group because they are hunters, with the rest being browsers. *Sardina pilchardus* seems closer to species of group 6, despite belonging to group 5.

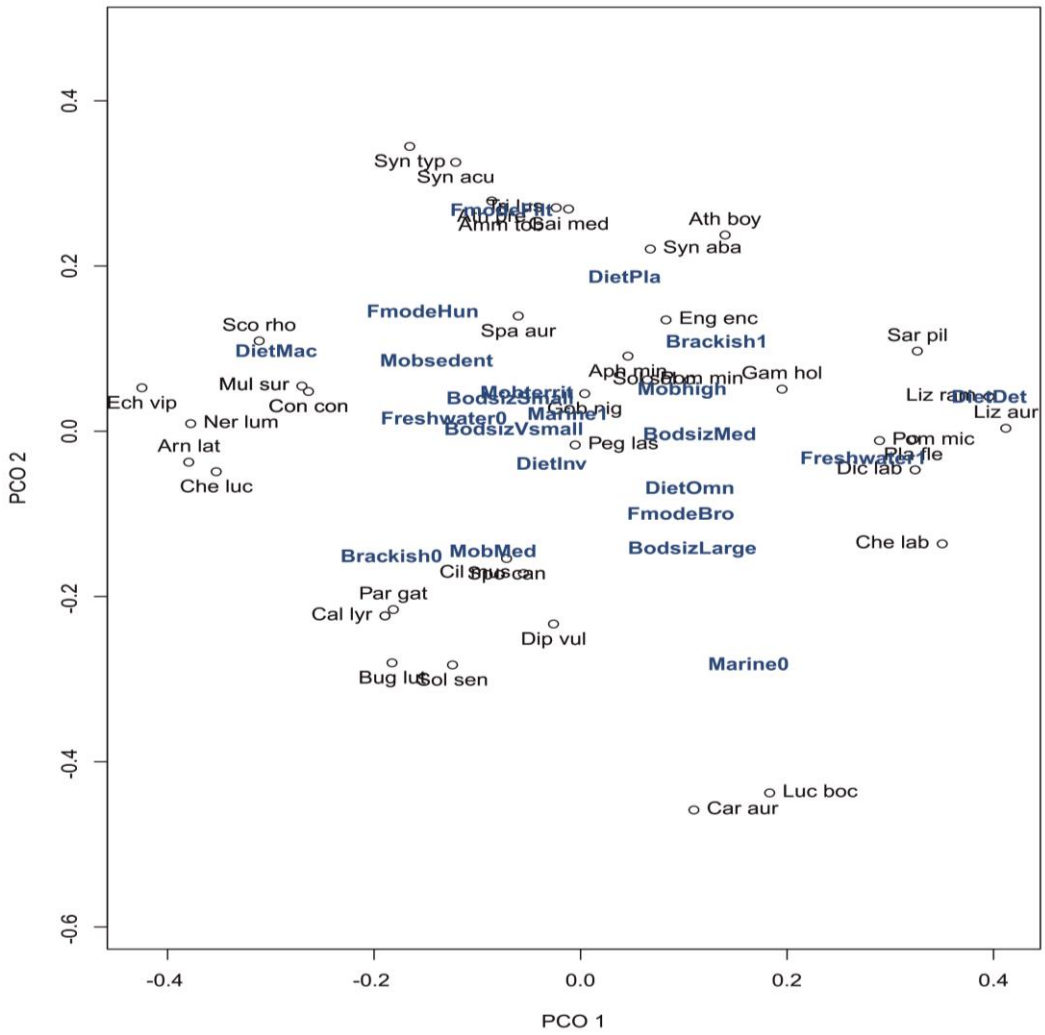


Figure 2.11 – Principal Coordinates Analysis comparing the fish assemblage of the Mondego estuary based on five traits: mobility, body size, diet, feeding mode and salinity preference (Mobility categories: Mobhigh – high; MobMed – medium; Mobterrit – territorial; Mobsedent – sedentary. Body size categories: BodsizLarge – large; BodsizMed – medium; BodsizSmall – small; BodsizVsmall – very small. Diet categories: DietPla – planktivorous; DietInv – invertebrate feeders; DietOmn – omnivorous; DietMac – macrocarnivorous; DietDet – detritivorous. Feeding mode categories: FmodeBro – browsers; FmodeFil – filterers; FmodeHun – hunters). Salinity preference categories are represented by Marine, Brackish and Freshwater (0 – no; 1 – yes). Species names are abbreviated. See full species names in Figure 2.7 (the abbreviation includes the first three letters of both genus and species).

PERMANOVA results showed that fish density, biomass and species richness of each functional group differed between stations (Tables 2.3-2.5), except for group 7 (in all measures) and group 4 (in species richness). Seasonal differences were only found for fish biomass and species richness of the functional groups, in spite of total density of species also showing differences among seasons. Inter-annual differences were found for all the measures, but not for all functional groups. Pair-wise comparisons (Tables 1-3 - Appendix II) for factor station revealed differences in fish metrics, but not between stations N1 and N2. Only species richness in group 5 and biomass in group 6 showed significant differences between these two stations. Pair-wise tests among seasons for biomass of species in each functional group were always significant, except between summer and spring, and autumn and winter for groups 2, 3 and 4.

Total biomass showed no differences between summer and autumn, summer and spring and autumn and spring. Seasonal differences were detected in pair-wise comparisons for total density of species between summer and autumn, summer and winter and summer and spring. Species richness showed no differences between seasons, except for groups 3, 4 and 5. For factor year, pair-wise tests showed differences for groups 5 and 6 for all measures.

Table 2.3 - PERMANOVA results for the differences in biomass of each functional group concerning factors season, station and year. For each test the mean, standard deviation (SD) and p-value - P(perm) - are represented (* for p-value < 0.05, NS for non-significant p-value).

		Total Biomass			Biomass Group 1			Biomass Group 2			Biomass Group 3			Biomass Group 4			Biomass Group 5			Biomass Group 6			Biomass Group 7		
Factor	Levels	Mean	SD	P(perm)	Mean	SD	P(perm	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)
Season	Autumn	708.861	805.650		11.358	58.213		53.409	129.513		33.622	75.947		6.499	22.972		235.388	436.074		368.342	515.408		0.243	1.514	
	Spring	559.051	737.145	*	7.789	38.028	NS	15.718	53.549	*	15.683	60.651	*	0.957	3.522	*	174.711	502.192	NS	344.177	453.358	NS	0.015	0.124	NS
	Summer	648.559	796.356		24.852	87.391		32.781	77.751		13.336	36.505		5.820	27.115		213.640	406.955		336.872	577.564		21.257	219.018	
	Winter	699.237	1163.487		13.005	43.629		71.152	255.752		35.597	92.330		5.429	20.237		210.649	500.437		336.944	551.996		26.461	227.380	
Station	M	1338.798	1323.770		53.182	116.033		138.915	274.146		77.600	122.551		15.613	41.249		636.637	822.854		416.852	685.999		0.000	0.000	
	N1	710.714	549.085		15.050	59.296		32.468	81.210		16.223	41.649		1.978	14.205		234.664	270.981		384.928	443.070		25.403	222.909	
	N2	808.529	829.928	*	0.001	0.007	*	1.959	10.009	*	0.649	3.922	*	0.378	2.479	*	104.510	190.512	*	669.918	672.670	*	31.115	265.813	NS
	S1	111.521	216.056		3.009	11.595		15.713	51.984		17.534	38.787		0.675	2.681		18.970	55.595		55.620	127.324		0.000	0.000	
	S2	307.915	293.957		1.203	6.763		15.734	39.675		5.567	27.846		4.420	9.326		50.131	85.156		230.325	247.072		0.535	2.643	
Year	2003	1166.775	1361.309		44.805	114.662		122.725	209.605		30.044	75.924		4.917	19.847		239.918	480.065		658.986	848.009		65.379	386.534	
	2004	828.910	1054.330		6.792	27.725		49.040	141.597		30.346	93.020		1.965	5.038		158.542	341.806		581.800	874.983		0.424	2.331	
	2005	609.695	888.169		9.403	38.338		44.616	221.654		41.880	88.698		16.367	41.864		245.724	700.232		251.705	283.042		0.000	0.000	
	2006	474.688	777.436		6.073	32.821		57.873	189.076		29.195	80.284		2.129	10.500		134.634	324.333		244.785	356.842		0.000	0.000	
	2007	553.084	758.018		34.862	93.361		22.789	50.876		23.138	70.795		7.216	26.385		284.229	540.868		180.230	267.653		0.620	2.685	
	2008	747.163	701.165	*	18.263	67.641	NS	10.192	35.590	*	18.366	32.511	NS	1.350	4.781	*	300.766	444.653	*	398.227	578.350	*	0.000	0.000	NS
	2009	949.499	715.143		11.078	35.481		33.107	87.640		35.473	81.588		0.952	3.303		344.976	523.867		523.912	467.438		0.000	0.000	
	2010	719.327	1051.507		24.884	119.910		22.082	63.419		12.248	35.540		0.053	0.271		329.504	583.873		330.081	485.418		0.476	2.473	
	2011	360.434	346.044		5.576	14.750		21.828	53.805		6.046	21.659		0.942	2.903		135.742	183.356		190.292	253.141		0.008	0.048	
	2012	313.624	393.702		5.432	14.897		26.979	75.565		3.244	13.042		4.985	21.002		89.359	151.034		183.626	278.582		0.000	0.000	
	2013	494.245	558.693		1.429	5.714		15.824	56.774		10.155	26.045		0.89	2.5038		85.527	163.985		322.689	427.208		57.732	335.420	

Table 2.4 - PERMANOVA results for the differences in density of each functional group concerning factors season, station and year. For each test the mean, standard deviation (SD) and p-value - P(perm) - are represented (* for p-value < 0.05, NS for non-significant p-value).

		Total Density			Density Group 1			Density Group 2			Density Group 3			Density Group 4			Density Group 5			Density Group 6			Density Group 7		
Factor	Levels	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)
Season	Autumn	29.361	49.513	*	0.298	0.936	NS	1.414	3.069	NS	0.570	1.261	NS	1.143	4.864	NS	6.091	9.580	NS	19.823	43.647	NS	0.021	0.145	NS
	Spring	25.703	37.184		0.434	0.969		0.572	2.257		0.261	0.767		0.367	1.045		6.939	12.241		17.119	31.652		0.010	0.076	
	Summer	42.791	55.986		0.532	1.247		1.886	4.734		0.387	0.704		1.092	4.432		11.734	25.218		27.074	45.531		0.087	0.539	
	Winter	19.688	21.833		0.327	0.981		0.923	2.771		0.440	0.971		1.078	3.328		4.163	8.108		12.744	16.080		0.012	0.073	
Station	M	30.396	36.700		1.068	1.703		2.658	5.061		0.938	1.405		0.384	0.969		17.514	28.488		7.835	10.138		0.000	0.000	
	N1	25.787	27.516		0.208	0.524		1.037	2.534		0.295	0.477		0.062	0.167		7.376	11.329		16.805	23.914		0.005	0.046	
	N2	24.352	25.951	*	0.010	0.058	*	0.106	0.378	*	0.015	0.092	*	0.023	0.101	*	3.429	7.420	*	20.731	22.344	*	0.037	0.153	NS
	S1	7.291	8.405		0.235	0.596		0.669	1.799		0.591	1.232		0.165	0.466		2.170	5.455		3.462	4.074		0.000	0.000	
	S2	62.264	74.796		0.479	1.132		1.624	4.400		0.214	0.540		3.771	7.569		6.967	11.774		49.081	66.901		0.129	0.633	
Year	2003	70.198	97.154		0.651	1.722		4.280	5.447		0.266	0.603		1.634	5.849		10.967	21.865		52.269	89.982		0.130	0.622	
	2004	27.685	26.549		0.413	1.137		0.912	2.253		0.543	0.958		0.769	2.067		4.849	10.006		20.078	23.756		0.121	0.682	
	2005	18.937	23.051		0.497	1.220		0.582	1.852		0.788	1.164		2.974	7.784		2.813	5.796		11.283	16.356		0.000	0.000	
	2006	24.679	33.495		0.066	0.176		0.804	1.741		0.428	0.880		0.473	2.017		5.565	10.969		17.343	28.981		0.000	0.000	
	2007	13.007	9.937		0.565	1.163		0.532	1.181		0.178	0.429		0.474	1.125		3.555	4.038		7.674	8.967		0.029	0.125	
	2008	20.003	18.026	*	0.629	1.437	*	0.133	0.291	*	0.563	0.860	*	0.034	0.104	NS	4.018	5.196	*	14.627	18.019	*	0.000	0.000	NS
	2009	25.324	16.862		0.306	0.530		0.932	2.357		0.764	1.935		0.240	1.008		6.278	7.462		16.805	15.639		0.000	0.000	
	2010	28.322	45.895		0.425	0.690		2.596	6.700		0.351	0.776		0.112	0.515		15.792	37.307		8.997	12.020		0.049	0.257	
	2011	53.397	66.637		0.298	0.437		1.525	5.131		0.161	0.339		0.938	2.342		18.672	24.488		31.783	53.372		0.021	0.118	
	2012	30.140	32.951		0.475	1.277		0.531	1.892		0.092	0.215		0.352	0.728		8.722	13.042		19.967	25.395		0.000	0.000	
	2013	26.778	28.414		0.210	0.627		1.094	2.654		0.173	0.375		0.252	0.746		6.334	7.643		18.675	23.892		0.040	0.133	

Table 2.5 - PERMANOVA results for the differences in species number of each functional group concerning factors season, station and year. For each test the mean, standard deviation (SD) and p-value - P(perm) - are represented (* for p-value < 0.05, NS for non-significant p-value).

		Total Species Number			Species Number Group 1			Species Number Group 2			Species Number Group 3			Species Number G4			Species Number Group 5			Species Number Group 6			Species Number Group 7		
Factor	Levels	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)
Season	Autumn	5.394	2.594	NS	0.269	0.507	NS	0.538	0.736	NS	0.625	0.905	*	0.260	0.540	*	1.481	0.892	*	2.192	1.062	NS	0.029	0.168	NS
	Spring	5.396	2.534		0.436	0.670		0.337	0.605		0.257	0.577		0.238	0.451		1.644	1.101		2.465	1.188		0.020	0.140	
	Summer	5.972	2.548		0.477	0.647		0.642	0.687		0.431	0.599		0.404	0.640		1.587	1.038		2.394	1.147		0.037	0.189	
	Winter	4.838	3.694		0.311	0.681		0.270	0.580		0.527	0.996		0.378	0.753		1.081	1.107		2.243	1.168		0.027	0.163	
Station	M	7.833	3.481	*	0.910	0.871	*	0.897	0.847	*	1.038	1.110	*	0.397	0.631	NS	2.359	0.967	*	2.231	1.092	*	0.000	0.000	NS
	N1	6.013	2.484		0.351	0.580		0.610	0.746		0.455	0.619		0.143	0.352		1.727	0.853		2.714	1.037		0.013	0.114	
	N2	4.054	1.820		0.014	0.116		0.122	0.329		0.027	0.163		0.068	0.253		0.797	0.702		2.959	1.176		0.068	0.253	
	S1	3.603	1.889		0.256	0.468		0.359	0.581		0.538	0.784		0.167	0.375		1.026	0.925		1.256	0.746		0.000	0.000	
	S2	5.679	1.903		0.346	0.479		0.321	0.470		0.210	0.467		0.778	0.837		1.444	1.000		2.519	0.808		0.062	0.242	
Year	2003	6.771	3.209	NS	0.600	0.775	*	1.000	0.728	NS	0.457	0.852	NS	0.343	0.539	NS	1.600	1.063	*	2.714	1.341	*	0.057	0.236	NS
	2004	5.641	3.376		0.462	0.822		0.462	0.682		0.564	0.852		0.333	0.701		1.256	1.208		2.513	1.144		0.051	0.223	
	2005	5.421	2.994		0.368	0.587		0.333	0.636		0.684	0.929		0.596	0.842		1.281	1.082		2.158	1.115		0.000	0.000	
	2006	4.875	3.337		0.200	0.564		0.475	0.816		0.425	0.747		0.325	0.656		1.425	1.238		2.025	1.050		0.000	0.000	
	2007	4.618	2.283		0.441	0.824		0.382	0.652		0.324	0.684		0.265	0.448		1.147	0.821		2.000	0.888		0.059	0.239	
	2008	4.950	2.012		0.350	0.587		0.250	0.550		0.700	0.865		0.100	0.308		1.250	0.786		2.300	1.261		0.000	0.000	
	2009	5.914	2.694		0.371	0.490		0.400	0.604		0.629	1.031		0.171	0.453		1.543	0.950		2.800	1.052		0.000	0.000	
	2010	5.185	2.883		0.444	0.641		0.630	0.792		0.407	0.694		0.074	0.267		1.407	0.971		2.185	1.360		0.037	0.192	
	2011	6.031	2.416		0.500	0.508		0.406	0.560		0.250	0.440		0.406	0.665		1.969	1.121		2.469	1.164		0.031	0.177	
	2012	4.914	2.513		0.286	0.519		0.343	0.539		0.171	0.382		0.314	0.471		1.743	1.010		2.057	1.027		0.000	0.000	
	2013	5.471	1.973		0.176	0.387		0.441	0.561		0.324	0.638		0.235	0.431		1.706	0.799		2.500	0.992		0.088	0.288	

The results of the GLM showed that biomass, density and number of species of the several trait categories were slightly related to the analyzed continuous environmental variables (Tables 2.6-2.8 and Appendix III). Many of the traits were positively linked with temperature (but negatively for detritivorous and hunters) and salinity (but negatively for detritivorous and freshwater species), and were negatively linked with oxygen (positively for territorial species and filterers). Smaller and larger species seem to respond differently to environmental variables: smaller increasing with salinity and decreasing with temperature, and the opposite for the larger. However, both respond negatively to oxygen. Relationships with runoff, precipitation and NAO index were very poor.

In contrast, the variation of fishes' traits was strongly related to station and also, though less, to year and showed a small relationship with season for all the measures used (i.e. biomass, density and number of species) (Tables 2.6-2.8 and Appendix III). Generally, station M (with higher oxygen values) and S2 (with higher temperature) showed higher density of traits, as well as number of species with those traits. The biomass of traits varied between stations, with higher values generally present at stations M and N1 and lower values at station S1. Mobility was the most responding trait to season, with sedentary species richness and density decreasing in autumn (with lower temperature) and medium mobility species number and biomass increasing in autumn (with lower oxygen and higher salinity). Species with very small body size (for density and biomass), hunters (for density and number of species) and freshwater species (for density and number of species) were generally more important in spring and summer and lower in autumn. Hunter species respond positively to salinity, which shows higher values in summer. As for factor year, 2003 was by far the year with the highest biomass, density and number of species, followed by 2009 and 2011. The years of 2006, 2007 and 2008 generally showed the lowest values for traits measures (see Appendix III).

Table 2.6 - Generalized Linear Model (GLM) fitted to the response of traits (in biomass) to environmental variables. The table presents for each variable: Sig - the significance of the variable in a full Generalized Linear Model (* p-value < 0.05), % - percentage of variance explained by the variable and C - coefficient signal (+ or -) estimated in Hierarchical Partition of Variance. Two alternative models were fitted for each trait: with and without factors (season, station, year), represented by A and B, respectively.

Trait		Salinity			Temperature			Oxygen			Runoff			Precipitation			NAO index			Season			Station			Year			%
		%	C	Sig	%	C	Sig	%	C	Sig	%	C	Sig	%	C	Sig	%	C	Sig	%	C	Sig	%	C	Sig	%	C	Sig	
High mobility	A	0.4	(+)		0.3	(+)		0.6	(-)		0.1	(+)		0.2	(+)		0.1	(-)		0.6	na		35.5	na	*	8.1	na	*	45.8
	B	0.6	(-)		0.0	(-)		0.4	(-)		0.2	(-)		0.3	(+)		0.1	(+)		na	na	na	na	na	na	na	na	na	1.6
Medium mobility	A	2.3	(-)		0.5	(+)		0.5	(-)	*	0.3	(+)		0.3	(-)		1.2	(+)		1.6	na	*	19.9	na	*	3.1	na	*	29.5
	B	4.9	(+)	*	0.6	(-)		0.2	(-)		0.3	(+)		0.1	(+)		1.6	(+)	*	na	na	na	na	na	na	na	na	na	7.6
Sedentary	A	0.3	(-)		0.5	(+)		0.3	(-)		0.2	(-)		0.1	(+)		0.0	(+)		0.9	na		1.9	na	*	2.6	na	*	6.8
	B	0.1	(-)		0.4	(+)		0.1	(-)		0.1	(-)		0.1	(-)		0.0	(+)		na	na	na	na	na	na	na	na	na	0.8
Territorial	A	1.3	(+)		0.7	(+)		0.1	(-)		0.2	(+)		0.5	(+)		0.3	(+)		0.9	na		7.8	na	*	3.8	na	*	15.6
	B	2.4	(+)	*	1.1	(+)	*	0.2	(+)		0.5	(+)		0.7	(+)	*	0.4	(+)		na	na	na	na	na	na	na	na	na	5.2
Large b.size	A	0.7	(+)		0.5	(+)		1.7	(-)	*	0.1	(+)		0.5	(+)		0.5	(+)		0.4	na		12.3	na	*	10.5	na	*	27.2
	B	1.3	(-)		0.7	(+)		1.6	(-)		0.1	(-)		0.8	(+)		0.8	(+)		na	na	na	na	na	na	na	na	na	5.2
Medium b.size	A	0.3	(+)		0.3	(+)		0.1	(-)		0.1	(+)		0.1	(-)		0.0	(-)		0.9	na		34.2	na	*	6.3	na	*	42.3
	B	0.2	(-)		0.1	(-)		0.0	(-)		0.1	(-)		0.0	(-)		0.0	(-)		na	na	na	na	na	na	na	na	na	0.4
Small b.size	A	2.9	(-)		0.2	(+)		0.4	(-)		0.3	(-)		0.1	(-)		0.3	(+)		1.6	na	*	16.0	na	*	4.4	na		26.1
	B	5.4	(+)	*	0.0	(-)		0.2	(-)		0.3	(-)		0.1	(+)		0.4	(+)		na	na	na	na	na	na	na	na	na	6.4
Very small b.size	A	2.8	(-)		0.5	(-)		0.8	(-)		0.3	(-)		0.6	(-)		0.3	(+)		0.4	na		33.5	na	*	8.4	na	*	47.6
	B	4.2	(+)	*	0.4	(-)		1.2	(+)		0.2	(+)		0.2	(-)		0.3	(+)		na	na	na	na	na	na	na	na	na	6.5
Planktivorous	A	0.5	(+)		4.2	(+)	*	0.1	(-)		0.3	(-)		0.5	(+)		0.3	(-)		1.4	na		3.3	na	*	4.0	na	*	14.4
	B	0.8	(+)		4.6	(+)	*	0.1	(+)		0.3	(+)		0.6	(-)		0.4	(-)		na	na	na	na	na	na	na	na	na	6.9
Invertebrate feeder	A	0.4	(+)		0.4	(+)	*	0.5	(-)		0.1	(+)		0.2	(+)		0.1	(-)		0.7	na		32.9	na	*	8.2	na	*	43.4
	B	0.5	(-)		0.0	(+)		0.4	(-)		0.2	(-)		0.3	(+)		0.1	(+)		na	na	na	na	na	na	na	na	na	1.4
Omnivorous	A	0.5	(-)		0.5	(+)		1.0	(-)	*	0.1	(-)		0.1	(+)		0.7	(+)		1.8	na		10.8	na	*	8.2	na	*	23.6
	B	0.5	(+)		0.3	(+)		0.3	(-)		0.1	(+)		0.2	(+)		1.0	(+)	*	na	na	na	na	na	na	na	na	na	2.4
Macrocarnivorous	A	2.0	(-)		0.5	(-)		0.5	(-)		0.2	(-)		0.3	(-)		0.1	(-)		0.6	na		25.5	na	*	3.2	na	*	32.9
	B	3.6	(+)	*	0.3	(-)		0.6	(+)		0.2	(-)		0.2	(-)		0.1	(+)		na	na	na	na	na	na	na	na	na	4.9
Detritivorous	A	1.5	(+)		0.4	(-)		0.5	(-)		0.4	(+)		0.0	(-)		0.0	(+)		0.4	na		4.4	na	*	4.3	na	*	12.0
	B	3.0	(-)	*	0.2	(-)		0.6	(-)		0.7	(+)		0.1	(-)		0.0	(-)		na	na	na	na	na	na	na	na	na	4.6
Browser	A	0.4	(+)		0.5	(+)	*	0.7	(-)		0.1	(+)		0.2	(+)		0.1	(-)		0.7	na		31.9	na	*	8.7	na	*	43.2
	B	0.6	(-)		0.1	(+)		0.5	(-)		0.1	(-)		0.4	(+)		0.1	(+)		na	na	na	na	na	na	na	na	na	1.8
Filterer	A	0.1	(+)		0.1	(+)		0.1	(+)		0.0	(+)		0.3	(-)		0.3	(+)		0.3	na		3.9	na	*	3.9	na	*	9.0
	B	0.1	(+)		0.1	(+)		0.2	(+)		0.0	(+)		0.2	(-)		0.3	(+)		na	na	na	na	na	na	na	na	na	0.8
Hunter	A	2.1	(-)		0.8	(-)		0.5	(-)		0.3	(-)		0.3	(-)		0.3	(+)		1.6	na		25.5	na	*	4.8	na	*	36.2
	B	3.6	(+)	*	0.7	(-)		0.4	(+)		0.3	(-)		0.2	(-)		0.3	(+)		na	na	na	na	na	na	na	na	na	5.6
Marine	A	0.2	(+)		0.4	(+)		0.6	(-)	*	0.1	(+)		0.2	(+)		0.1	(-)		0.7	na		35.3	na	*	8.1	na	*	45.5
	B	0.3	(-)		0.0	(-)		0.4	(-)		0.1	(-)		0.3	(+)		0.1	(+)		na	na	na	na	na	na	na	na	na	1.2
Brackish	A	0.6	(+)		0.3	(+)		0.5	(-)		0.1	(+)		0.1	(+)		0.1	(-)		0.5	na		32.2	na	*	8.4	na	*	42.8
	B	1.0	(-)		0.0	(+)		0.5	(-)		0.1	(-)		0.2	(+)		0.1	(+)		na	na	na	na	na	na	na	na	na	1.8
Freshwater	A	4.2	(+)		0.2	(+)		1.8	(-)	*	0.1	(+)		0.4	(+)		0.1	(+)		0.3	na		17.2	na	*	10.4	na	*	34.6
	B	8.3	(-)	*	0.2	(+)		2.1	(-)		0.3	(-)		0.4	(+)		0.2	(+)		na	na	na	na	na	na	na	na	na	11.5

Table 2.7 - Generalized Linear Model (GLM) fitted to the response of traits (in density) to environmental variables. The table presents for each variable: Sig - the significance of the variable in a full Generalized Linear Model (* p-value < 0.05), % - percentage of variance explained by the variable and C - coefficient signal (+ or -) estimated in Hierarchical Partition of Variance. Two alternative models were fitted for each trait: with and without factors (season, station, year), represented by A and B, respectively.

Trait		Salinity			Temperature			Oxygen			Runoff			Precipitation			NAO index			Season			Station			Year			%
		%	C	Sig	%	C	Sig	%	C	Sig	%	C	Sig	%	C	Sig	%	C	Sig	%	C	Sig	%	C	Sig	%	C	Sig	
High mobility	A	0.3	(-)		3.6	(+)		2.3	(-)	*	0.3	(-)		0.3	(-)		0.2	(+)		1.9	na		21.6	na	*	11.1	na	*	41.5
	B	0.4	(-)		7.0	(+)	*	3.1	(-)	*	0.3	(+)		0.3	(-)		0.2	(+)		na	na	na	na	na	na	na	na	na	11.4
Medium mobility	A	4.2	(-)		0.3	(+)		0.7	(-)	*	0.1	(+)		0.2	(-)		0.4	(+)		0.9	na		19.9	na	*	4.0	na	*	30.7
	B	8.8	(+)	*	0.3	(-)		0.2	(-)		0.2	(+)		0.1	(+)		0.5	(+)		na	na	na	na	na	na	na	na	na	10.1
Sedentary	A	1.1	(+)		2.7	(+)		0.3	(-)		0.2	(-)		0.3	(+)		0.2	(+)		2.3	na	*	20.4	na	*	5.4	na	*	32.8
	B	1.8	(+)	*	5.2	(+)	*	0.4	(-)		0.3	(+)		0.3	(+)		0.3	(+)		na	na	na	na	na	na	na	na	na	8.3
Territorial	A	1.3	(+)		0.8	(+)		0.3	(+)		0.1	(-)		0.3	(+)		0.1	(+)		2.0	na		10.7	na	*	4.9	na	*	20.5
	B	2.1	(+)	*	1.4	(+)	*	0.4	(+)		0.1	(+)		0.6	(+)	*	0.1	(+)		na	na	na	na	na	na	na	na	na	4.7
Large b.size	A	0.6	(+)		4.2	(+)		5.5	(-)	*	0.1	(+)		0.3	(+)		0.2	(+)		1.6	na		21.2	na	*	13.2	na	*	46.9
	B	0.8	(-)		7.8	(+)	*	7.2	(-)	*	0.3	(+)		0.9	(+)	*	0.5	(+)		na	na	na	na	na	na	na	na	na	17.5
Medium b.size	A	3.0	(-)		1.7	(+)	*	1.3	(-)		0.5	(+)		0.1	(+)		0.1	(-)		0.7	na		13.2	na	*	11.3	na	*	31.8
	B	5.0	(-)	*	2.4	(+)	*	2.0	(-)		0.3	(+)		0.1	(-)		0.2	(-)		na	na	na	na	na	na	na	na	na	10.0
Small b.size	A	2.9	(+)		0.5	(-)		0.6	(-)	*	0.6	(-)		0.2	(-)		0.1	(+)		0.9	na		27.3	na	*	7.4	na	*	40.5
	B	5.3	(+)	*	0.6	(+)		0.5	(-)		0.5	(+)		0.1	(+)		0.1	(+)		na	na	na	na	na	na	na	na	na	7.0
Very small b.size	A	1.5	(-)		0.9	(+)		0.1	(-)		0.8	(-)		1.6	(-)		0.5	(+)		1.9	na	*	16.4	na	*	12.9	na	*	36.5
	B	1.7	(+)	*	1.8	(+)	*	0.0	(-)		0.5	(+)		1.6	(-)		0.3	(+)		na	na	na	na	na	na	na	na	na	5.9
Planktivorous	A	0.8	(+)		1.8	(+)	*	0.3	(+)		0.5	(-)		0.3	(+)		0.3	(-)		1.6	na		3.8	na	*	4.5	na		13.8
	B	1.1	(+)		2.3	(+)	*	0.7	(+)		0.6	(-)		0.4	(-)		0.4	(-)		na	na	na	na	na	na	na	na	na	5.4
Invertebrate feeder	A	0.3	(-)		3.6	(+)		2.4	(-)	*	0.3	(-)		0.4	(-)		0.2	(+)		1.8	na		20.8	na	*	9.8	na	*	39.7
	B	0.4	(-)		7.1	(+)	*	3.4	(-)	*	0.4	(+)		0.4	(-)		0.2	(+)		na	na	na	na	na	na	na	na	na	11.8
Omnivorous	A	1.5	(-)		1.9	(+)	*	0.9	(-)		0.3	(-)		0.1	(-)		0.1	(+)		2.6	na		10.9	na	*	11.9	na	*	30.1
	B	2.4	(+)	*	2.8	(+)	*	0.5	(-)		0.3	(+)		0.1	(+)		0.1	(+)		na	na	na	na	na	na	na	na	na	6.2
Macrocarnivorous	A	4.0	(+)		0.2	(+)		0.5	(-)	*	0.2	(+)		0.2	(-)		0.1	(+)		0.9	na		18.0	na	*	2.9	na	*	27.0
	B	7.9	(+)	*	0.2	(+)		0.1	(-)		0.3	(+)		0.2	(-)		0.1	(+)		na	na	na	na	na	na	na	na	na	8.8
Detritivorous	A	1.0	(-)		1.0	(-)	*	0.3	(-)		1.2	(+)		0.2	(-)		0.0	(-)		0.4	na		9.1	na	*	3.5	na		16.7
	B	1.5	(-)		0.2	(-)		0.5	(-)		1.8	(+)	*	0.3	(+)		0.1	(-)		na	na	na	na	na	na	na	na	na	4.4
Browser	A	0.3	(-)		4.1	(+)		2.1	(-)		0.3	(-)		0.3	(-)		0.2	(+)		1.9	na		19.2	na	*	11.6	na	*	40.0
	B	0.4	(-)		7.9	(+)	*	3.0	(-)	*	0.3	(+)		0.3	(-)		0.2	(+)		na	na	na	na	na	na	na	na	na	12.1
Filterer	A	0.2	(+)		0.1	(+)		1.0	(+)		0.2	(-)		0.1	(-)		0.1	(-)		0.6	na		2.6	na		3.0	na		7.8
	B	0.2	(+)		0.1	(-)		1.7	(+)	*	0.4	(-)		0.1	(-)		0.1	(-)		na	na	na	na	na	na	na	na	na	2.5
Hunter	A	3.0	(+)		0.5	(-)	*	0.8	(-)	*	0.2	(-)		0.3	(-)		0.7	(+)		1.1	na	*	24.1	na	*	7.1	na	*	37.8
	B	5.2	(+)	*	0.1	(+)		0.5	(-)		0.2	(+)		0.3	(-)		0.6	(+)		na	na	na	na	na	na	na	na	na	6.9
Marine	A	0.2	(-)		3.7	(+)		2.2	(-)	*	0.3	(-)		0.3	(-)		0.2	(+)		1.9	na		21.7	na	*	10.6	na	*	41.2
	B	0.2	(-)		7.2	(+)	*	3.0	(-)	*	0.3	(+)		0.3	(-)		0.2	(+)		na	na	na	na	na	na	na	na	na	11.3
Brackish	A	0.3	(-)		3.5	(+)		2.3	(-)	*	0.3	(-)		0.4	(-)		0.2	(+)		1.8	na		22.5	na	*	10.1	na	*	41.3
	B	0.5	(-)		6.9	(+)	*	3.2	(-)	*	0.3	(+)		0.4	(-)		0.2	(+)		na	na	na	na	na	na	na	na	na	11.5
Freshwater	A	1.7	(-)		3.3	(+)		2.7	(-)		0.2	(-)		0.1	(+)		0.5	(+)		1.5	na	*	26.0	na	*	7.8	na	*	43.6
	B	3.1	(-)	*	6.4	(+)	*	3.9	(-)	*	0.3	(+)		0.2	(+)		0.6	(+)		na	na	na	na	na	na	na	na	na	14.3

Table 2.8 - Generalized Linear Model (GLM) fitted to the response of traits (in number of species) to environmental variables. The table presents for each variable: Sig - the significance of the variable in a full Generalized Linear Model (* p-value < 0.05), % - percentage of variance explained by the variable and C - coefficient signal (+ or -) estimated in Hierarchical Partition of Variance. Two alternative models were fitted for each trait: with and without factors (season, station, year), represented by A and B, respectively.

Trait		Salinity			Temperature			Oxygen			Runoff			Precipitation			NAO index			Season			Station			Year			%
		%	C	Sig	%	C	Sig	%	C	Sig	%	C	Sig	%	C	Sig	%	C	Sig	%	C	Sig	%	C	Sig	%	C	Sig	
High mobility	A	0.3	(-)		0.7	(+)		1.3	(-)	*	0.6	(-)		0.5	(-)		0.0	(+)		0.9	na		30.2	na	*	6.2	na	*	40.6
	B	0.3	(+)		0.7	(+)		0.8	(-)		0.7	(-)		0.4	(-)		0.0	(+)		na	na	na	na	na	na	na	na	na	2.9
Medium mobility	A	3.7	(-)		0.6	(+)		0.6	(-)		0.2	(+)		0.1	(-)		1.0	(+)		1.4	na	*	22.3	na	*	3.3	na	*	33.3
	B	7.9	(+)	*	1.0	(-)		0.3	(-)		0.3	(+)		0.1	(+)		1.2	(+)	*	na	na	na	na	na	na	na	na	na	10.9
Sedentary	A	1.8	(+)		3.1	(+)	*	0.6	(-)		0.3	(-)		0.4	(+)		0.2	(+)		2.5	na	*	15.2	na	*	4.9	na	*	29.0
	B	2.9	(+)	*	5.1	(+)	*	0.5	(-)		0.4	(+)		0.3	(+)		0.1	(+)		na	na	na	na	na	na	na	na	na	9.3
Territorial	A	1.8	(+)		0.2	(+)		1.1	(+)		0.2	(-)		0.4	(+)		0.4	(+)		2.0	na	*	8.0	na	*	3.0	na	*	16.9
	B	3.0	(+)	*	0.5	(+)		1.8	(+)	*	0.1	(+)		0.6	(+)	*	0.4	(+)		na	na	na	na	na	na	na	na	na	6.3
Large b.size	A	0.6	(-)		2.2	(+)		2.8	(-)	*	0.6	(-)		0.1	(-)		0.5	(+)		1.0	na		9.8	na	*	8.2	na	*	25.7
	B	1.1	(-)		3.0	(+)	*	3.0	(-)	*	0.6	(-)		0.1	(+)		0.7	(+)		na	na	na	na	na	na	na	na	na	8.5
Medium b.size	A	0.3	(+)		0.6	(+)		0.3	(-)		0.2	(+)		0.2	(-)		0.4	(+)		0.7	na		23.2	na	*	6.5	na	*	32.2
	B	0.3	(+)		0.5	(+)		0.1	(-)		0.1	(+)		0.2	(-)		0.4	(+)		na	na	na	na	na	na	na	na	na	1.6
Small b.size	A	3.6	(-)		0.1	(+)		0.6	(-)	*	0.9	(-)		0.1	(-)		0.0	(+)		0.8	na		20.4	na	*	4.9	na	*	31.5
	B	6.1	(+)	*	0.1	(+)		0.1	(-)		0.7	(-)		0.1	(+)		0.0	(-)		na	na	na	na	na	na	na	na	na	7.2
Very small b.size	A	4.6	(-)		0.3	(-)		0.8	(-)		0.2	(-)		0.4	(-)		0.1	(-)		0.4	na		26.7	na	*	6.4	na	*	39.9
	B	8.0	(+)	*	0.2	(-)		1.0	(+)		0.2	(+)		0.2	(+)		0.1	(+)		na	na	na	na	na	na	na	na	na	9.6
Planktivorous	A	1.1	(+)		1.8	(+)	*	0.2	(+)		0.4	(-)		0.4	(-)		0.4	(-)		1.9	na	*	3.7	na	*	5.8	na	*	15.6
	B	1.3	(+)		2.2	(+)	*	0.5	(+)		0.5	(-)		0.5	(-)		0.5	(-)		na	na	na	na	na	na	na	na	na	5.4
Invertebrate feeder	A	0.8	(-)		0.4	(+)		0.8	(-)	*	0.5	(-)		0.1	(-)		0.4	(+)		0.3	na		26.5	na	*	3.7	na	*	33.5
	B	1.4	(+)	*	0.2	(+)		0.4	(-)		0.6	(-)		0.1	(+)		0.5	(+)		na	na	na	na	na	na	na	na	na	3.2
Omnivorous	A	1.2	(-)		1.1	(+)		1.6	(-)	*	0.2	(-)		0.3	(-)		0.1	(+)		3.0	na		10.8	na	*	5.4	na	*	23.7
	B	1.8	(+)	*	1.6	(+)	*	1.0	(-)		0.2	(+)		0.1	(+)		0.1	(+)		na	na	na	na	na	na	na	na	na	4.8
Macrocarnivorous	A	4.1	(+)		0.2	(+)		0.5	(-)		0.2	(+)		0.2	(-)		0.3	(+)		0.8	na		22.5	na	*	4.0	na	*	32.7
	B	7.1	(+)	*	0.1	(+)		0.7	(+)		0.2	(+)		0.2	(+)		0.4	(+)		na	na	na	na	na	na	na	na	na	8.6
Detritivorous	A	1.6	(-)		0.4	(-)		0.4	(-)		0.3	(-)		0.1	(-)		0.0	(-)		0.6	na		9.2	na	*	5.0	na		17.5
	B	3.1	(-)	*	0.0	(-)		0.6	(-)		0.5	(+)		0.2	(-)		0.0	(-)		na	na	na	na	na	na	na	na	na	4.4
Browser	A	0.5	(-)		1.6	(+)	*	0.8	(-)	*	0.3	(-)		0.2	(-)		0.2	(+)		0.8	na		24.4	na	*	4.8	na	*	33.5
	B	0.8	(+)		1.5	(+)	*	0.5	(-)		0.3	(+)		0.1	(+)		0.3	(+)		na	na	na	na	na	na	na	na	na	3.5
Filterer	A	0.4	(+)		0.1	(+)		0.7	(+)		0.1	(+)		0.2	(-)		0.0	(-)		0.4	na		4.4	na		4.5	na	*	10.7
	B	0.3	(+)		0.0	(+)		1.2	(+)		0.1	(-)		0.2	(-)		0.0	(+)		na	na	na	na	na	na	na	na	na	1.8
Hunter	A	3.7	(+)		0.2	(-)		0.8	(-)	*	0.5	(-)		0.3	(-)		0.3	(+)		1.4	na	*	23.0	na	*	6.2	na	*	36.4
	B	6.4	(+)	*	0.1	(-)		0.1	(-)		0.4	(+)		0.3	(-)		0.2	(+)		na	na	na	na	na	na	na	na	na	7.6
Marine	A	1.9	(-)		0.6	(+)		0.9	(-)	*	0.4	(-)		0.3	(-)		0.3	(+)		0.6	na		30.8	na	*	4.9	na	*	40.8
	B	3.3	(+)	*	0.6	(+)		0.3	(-)		0.4	(+)		0.2	(+)		0.3	(+)		na	na	na	na	na	na	na	na	na	5.2
Brackish	A	0.3	(-)		1.1	(+)		1.2	(-)	*	0.7	(-)		0.6	(-)		0.1	(+)		1.3	na	*	26.2	na	*	5.8	na	*	37.3
	B	0.2	(+)		1.7	(+)		1.0	(-)		0.7	(-)		0.6	(-)		0.2	(+)		na	na	na	na	na	na	na	na	na	4.3
Freshwater	A	3.0	(-)		1.0	(+)		2.9	(-)	*	0.4	(-)		1.1	(-)		0.5	(+)		1.3	na	*	20.6	na	*	5.8	na	*	36.5
	B	6.3	(-)	*	1.4	(+)		3.7	(-)	*	0.5	(-)		1.6	(-)	*	0.6	(+)		na	na	na	na	na	na	na	na	na	14.0

Functional redundancy of the total assemblage differed between stations (for both biomass and density, with higher redundancy in stations N1 and N2 and lower in M and S1) and also between seasons (in density, with higher redundancy in spring and autumn), but not years (Tables 2.9-2.10). Functional redundancy was null for functional group 7 whilst for functional groups 2, 3, 5 and 6 it varied with the tested factors, but differently and not necessarily for same measures (density and biomass): spatial variation was found for groups 5 (showing higher functional redundancy in stations M and S2) and 6 (presenting higher functional redundancy in stations N1 and N2); seasonal variation for groups 3 (with higher functional redundancy in autumn and winter) and 5 (with higher functional redundancy in autumn and spring); and inter-annual variation for groups 2, 5 and 6. The years showing higher functional redundancy were 2011 (for biomass of group 5), 2007 (for density of group 2) and 2009 (for density of group 6).

Table 2.9 - Functional redundancy (FR) by season, station and year for total biomass of species and for each functional group, represented by mean, standard-deviation (SD) and p-value from PERMANOVA (* for p-value < 0.05, NS for non-significant p-value).

		FR Total Biomass			FR Group 1			FR Group 2			FR Group 3			FR Group 4			FR Group 5			FR Group 6			FR Group 7		
Factor	Levels	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)
Season	Autumn	0.177	0.095	NS	0.014	0.038	NS	0.027	0.053	NS	0.064	0.110	*	0.029	0.078	NS	0.041	0.068	NS	0.120	0.135	NS	0	0	NS
	Spring	0.181	0.132		0.016	0.050		0.023	0.054		0.018	0.042		0.008	0.036		0.056	0.075		0.168	0.158		0	0	
	Summer	0.174	0.105		0.004	0.016		0.019	0.048		0.014	0.048		0.018	0.060		0.054	0.080		0.145	0.146		0	0	
	Winter	0.160	0.114		0.026	0.052		0.024	0.051		0.086	0.118		0.052	0.081		0.034	0.059		0.155	0.147		0	0	
Station	M	0.158	0.089	*	0.024	0.051	NS	0.044	0.068	NS	0.072	0.098	NS	0.020	0.057	NS	0.076	0.085	*	0.143	0.148	*	0	0	NS
	N1	0.207	0.105		0.013	0.041		0.024	0.047		0.017	0.058		0.000	0.000		0.040	0.068		0.184	0.149		0	0	
	N2	0.229	0.131		0.000	0.000		0.000	0.000		0.000	0.000		0.000	0.000		0.005	0.013		0.215	0.142		0	0	
	S1	0.106	0.101		0.000	0.000		0.013	0.035		0.042	0.107		0.000	0.000		0.037	0.063		0.060	0.121		0	0	
	S2	0.174	0.088		0.000	0.000		0.000	0.000		0.018	0.059		0.042	0.083		0.064	0.079		0.123	0.130		0	0	
Year	2003	0.182	0.100	NS	0.027	0.048	NS	0.035	0.053	NS	0.075	0.105	NS	0.007	0.024	NS	0.043	0.077	*	0.157	0.166	NS	0	0	NS
	2004	0.195	0.144		0.060	0.079		0.013	0.040		0.038	0.078		0.050	0.100		0.050	0.071		0.192	0.170		0	0	
	2005	0.172	0.128		0.000	0.002		0.038	0.065		0.051	0.092		0.044	0.075		0.020	0.039		0.153	0.165		0	0	
	2006	0.149	0.097		0.015	0.039		0.030	0.055		0.016	0.061		0.044	0.096		0.056	0.067		0.115	0.140		0	0	
	2007	0.137	0.082		0.024	0.057		0.031	0.062		0.034	0.064		0.000	0.000		0.017	0.033		0.122	0.159		0	0	
	2008	0.175	0.122		0.013	0.032		0.028	0.055		0.048	0.079		0.000	0.000		0.019	0.043		0.171	0.162		0	0	
	2009	0.207	0.114		0.000	0.000		0.020	0.048		0.114	0.160		0.046	0.102		0.034	0.056		0.173	0.130		0	0	
	2010	0.154	0.103		0.000	0.001		0.036	0.077		0.020	0.060		0.000	0.000		0.038	0.074		0.125	0.123		0	0	
	2011	0.203	0.102		0.000	0.000		0.002	0.007		0.000	0.000		0.020	0.066		0.068	0.077		0.166	0.143		0	0	
	2012	0.159	0.099		0.000	0.000		0.010	0.034		0.000	0.000		0.000	0.000		0.112	0.104		0.108	0.111		0	0	
	2013	0.183	0.103		0.000	0.000		0.000	0.001		0.026	0.041		0.000	0.000		0.057	0.078		0.126	0.117		0	0	

Table 2.10 - Functional redundancy (FR) by season, station and year for total density of species and for each functional group, represented by mean, standard-deviation (SD) and p-value from PERMANOVA (* for p-value < 0.05, NS for non-significant p-value).

		FR Total Density			FR Group 1			FR Group 2			FR Group 3			FR Group 4			FR Group 5			FR Group 6			FR Group 7		
Factor	Levels	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)	Mean	SD	P(perm)
Season	Autumn	0.218	0.089	*	0.019	0.055	NS	0.035	0.075	NS	0.076	0.123	NS	0.030	0.082	NS	0.098	0.108	*	0.174	0.136	NS	0	0	NS
	Spring	0.219	0.113		0.041	0.077		0.037	0.070		0.040	0.081		0.012	0.058		0.093	0.096		0.188	0.141		0	0	
	Summer	0.194	0.092		0.020	0.059		0.022	0.066		0.023	0.073		0.020	0.063		0.075	0.087		0.160	0.128		0	0	
	Winter	0.204	0.103		0.045	0.074		0.028	0.057		0.095	0.124		0.089	0.123		0.085	0.102		0.171	0.131		0	0	
Station	M	0.199	0.079	*	0.053	0.082	NS	0.056	0.088	NS	0.089	0.112	NS	0.036	0.093	NS	0.149	0.093	NS	0.166	0.137	*	0	0	NS
	N1	0.232	0.089		0.031	0.069		0.024	0.049		0.019	0.072		0.000	0.000		0.091	0.094		0.203	0.125		0	0	
	N2	0.247	0.128		0.000	0.000		0.000	0.000		0.000	0.000		0.000	0.000		0.021	0.062		0.232	0.134		0	0	
	S1	0.169	0.102		0.010	0.042		0.028	0.080		0.059	0.124		0.000	0.000		0.063	0.093		0.094	0.130		0	0	
	S2	0.200	0.073		0.000	0.000		0.000	0.000		0.030	0.082		0.051	0.097		0.085	0.094		0.163	0.111		0	0	
Year	2003	0.195	0.094	NS	0.061	0.082	NS	0.013	0.025	*	0.079	0.105	NS	0.025	0.083	NS	0.075	0.101	NS	0.161	0.136	*	0	0	NS
	2004	0.212	0.124		0.091	0.089		0.024	0.046		0.067	0.109		0.067	0.109		0.090	0.097		0.192	0.151		0	0	
	2005	0.209	0.103		0.011	0.048		0.050	0.090		0.068	0.106		0.058	0.099		0.078	0.092		0.183	0.155		0	0	
	2006	0.174	0.098		0.031	0.076		0.031	0.056		0.018	0.068		0.056	0.118		0.101	0.095		0.125	0.123		0	0	
	2007	0.196	0.081		0.064	0.104		0.071	0.116		0.075	0.140		0.000	0.000		0.073	0.108		0.155	0.139		0	0	
	2008	0.202	0.076		0.023	0.056		0.069	0.138		0.066	0.113		0.000	0.000		0.085	0.090		0.178	0.123		0	0	
	2009	0.251	0.087		0.000	0.000		0.035	0.084		0.105	0.158		0.049	0.110		0.107	0.102		0.220	0.113		0	0	
	2010	0.198	0.090		0.029	0.063		0.048	0.095		0.027	0.080		0.000	0.000		0.078	0.095		0.199	0.116		0	0	
	2011	0.214	0.100		0.000	0.000		0.015	0.053		0.000	0.000		0.025	0.082		0.076	0.090		0.162	0.126		0	0	
	2012	0.197	0.108		0.020	0.061		0.011	0.035		0.000	0.000		0.000	0.000		0.117	0.106		0.141	0.118		0	0	
	2013	0.249	0.089		0.000	0.000		0.008	0.030		0.055	0.106		0.000	0.000		0.088	0.102		0.197	0.132		0	0	

Discussion

In this study, the fish assemblage of the Mondego estuary was characterized in terms of its functional traits in order to assess its role in ecosystem functioning and services. This was accomplished with the identification of functional groups within the fish assemblage and a literature review of the links between functional traits of fish and ecosystem functioning and regulating ecosystem services. In our innovative approach we assessed how the studied fish assemblage potentially influences ecosystem functioning and services, its degree of resilience (i.e. measured through functional redundancy) and how it is regulated by environmental variables.

Literature review of links between fish functional traits and ecosystem functioning and services

The literature review allowed the identification of several links between fish traits and ecosystem services, and especially highlighted links for traits such as feeding mode and diet. It should be noted that the links of traits with two of the studied regulating ecosystem services were context-specific. Specifically, coastal protection and disturbance prevention showed different links with fish traits depending on habitat: biogenic habitats such as coral reefs (Cheal *et al.* 2010) or vegetated habitats like seagrass beds (Heck & Valentine 2006). Also, links with biological control were divided in food web control (separating bottom-up control, wasp-waist control from top-down control), control of diseases, control of invasions and control of algal blooms (Valiela *et al.* 1997; Caddy & Garibaldi 2000; Cury *et al.* 2003; Iguchi & Yodo 2004; Shahi *et al.* 2015).

The majority of evidence found in the literature was for indirect relationships between functional traits and the provision of ecosystem services. For example, herbivorous fish contribute to the control of establishment of macroalgae in coral reefs, thus enhancing the resilience of these natural barriers, which is essential for the service of coastal protection and disturbance prevention (Nyström *et al.* 2000; Hoey & Bellwood 2011; Pratchett *et al.* 2014). Nevertheless, direct connections were found, for example, between migrating fish species (with high mobility) and the regulation of linkages between ecosystems, such as estuaries and coastal zones (Deegan 1993; Holmlund & Hammer 1999).

Several traits (e.g. feeding modes such as browsing, grazing and filtering) can influence more than one ecosystem service (Andersson *et al.* 1978; Holmlund & Hammer 1999; Zhou *et al.* 1999; Xie & Liu 2001; Adámek & Maršálek 2013; Pratchett *et al.* 2014). Dietary traits generally influenced more than one ecosystem service, although showing different effects depending on the service, for example: macrocarnivorous fish showed a positive effect (enhancement) on top-down control (Carpenter *et al.* 1985) but a negative effect (diminution) on climate regulation (Schindler *et al.* 1997); while herbivorous fish enhanced waste treatment and assimilation (Han *et al.* 2015) but decreased nutrient cycling (Flecker *et al.* 2002). In contrast, omnivorous, detritivorous and planktivorous fish showed a positive effect in the provision of several ecosystem services, such as nutrient cycling, coastal protection and disturbance prevention and biological control (Andersson *et al.* 1978; Ruitton *et al.* 2000; Taylor *et al.* 2006; Vanni *et al.* 2008). Body size also had noteworthy connections to the provision of ecosystem services, showing different effects: for example smaller fish contribute highly to nutrient cycling because they have higher metabolic and excretion rates than larger fish, thus increasing nutrient concentrations in the water (Carpenter *et al.* 1992); meanwhile larger fish are effective in controlling smaller fish populations (biological control), (Strange *et al.* 1999; Woodward *et al.* 2005) and in the regulation of linkages between ecosystems since larger fish usually have wider home ranges and territories, thus being able to move over larger distances than smaller fish (Woodward *et al.* 2005). Overall, since these traits (i.e. feeding mode, diet and body size) show connections to ecosystem functioning and have also been used in several

functional characterization studies of fish and invertebrates (Bremner *et al.* 2003; Gerino *et al.* 2003; Auster & Link 2009; Baptista *et al.* 2015b; Costello *et al.* 2015), they apparently have significant potential in research about ecosystem functioning and services.

Functional groups of the fish assemblage in the Mondego estuary

Based on five functional traits (i.e. mobility, body size, diet, feeding mode and salinity preference), the fish assemblage of the Mondego estuary was characterized in seven functional groups (through cluster analysis). Considering that the seven functional groups in the fish assemblage display different combinations of traits, they can also have a different potential to contribute to specific ecosystem services, as shown by García-Llorente *et al.* (2011) for aquatic plant communities. Considering our literature review of links between functional traits and ecosystem functioning and services, the Mondego estuary fish assemblage includes a high number of functional groups that can enhance nutrient cycling, biological control and maintenance of sediment processes and sediment redistribution. Moreover, although waste treatment and assimilation and also climate regulation services seem to be less enhanced by this fish assemblage (since they are only promoted by group 5 and groups 5 and 6, respectively) the high number of species, abundance and biomass that represent functional groups 5 and 6 might be indicative of the high importance of this fish assemblage to these services. However, further research should quantify the contribution of fish assemblages to services in order to establish which are the most important and may require conservation efforts. The only regulating service not especially influenced by the Mondego estuary fish assemblage was air purification, because none of the species is herbivorous.

Variation and drivers of fish functional traits and groups in the Mondego estuary

Spatial differences of three measures (biomass, density and species richness) of each functional group (except group 7) among stations within the Mondego estuary are probably associated with environmental conditions. Higher values of these three measures for the majority of the functional groups at station M could be due to higher salinity and oxygen in this station (near the mouth of the estuary), given that this fish assemblage is mostly composed by marine species (Nyitrai *et al.* 2012; Baptista *et al.* 2015b). In contrast, station N2 - the most upstream and with lower salinities - showed the lowest values for groups 1, 2 and 3 (in all three measures) because these groups (particularly 2 and 3) are composed by markedly marine species. The lower biomass and density of groups 5 and 6 at station S1 may be explained by: lower temperature at this station for group 5, because it contains planktivorous species that are positively correlated to temperature (according to the GLM) and therefore may decrease at this station, with lower temperature; and higher salinity at this station for group 6, because it is an environmental variable negatively correlated to detritivorous species (present in group 6), according to the GLM, which may lead to their decrease at this station, presenting higher salinity. Also, Whitfield *et al.* (2006) showed that long-term effects of hyperhaline conditions (i.e. above 40) in estuaries are negative for detritivorous fish. However, this trend may need further investigation. Group 7 was the only group without significant differences between stations, probably because it is the smallest group regarding species number (with only three freshwater species), that showed the lowest biomass, density and species richness in the sampling period (2003-2013). This could have contributed to the absence of significant differences between stations. Moreover, there has been evidence of a progressive decrease of freshwater species within the section of the estuary covered by this monitoring program, due to an increase of salinity, mostly because of anthropogenic interventions, such as dredging activities (Leitão *et al.* 2007; Nyitrai *et al.* 2012).

In addition, the fish functional groups also showed seasonal variations. Seasonal differences in species richness for group 5 could be related to temperature, as the highest values were registered in spring and the lowest values in winter. Moreover, GLM showed a positive correlation between number of planktivorous species (present only in group 5) and temperature. However, differences in biomass of groups 2, 3 and 4 between seasons may have different reasons, such as recruitment dynamics (Akin *et al.* 2003), because these groups contain marine species which colonize estuaries as stragglers or frequently as migrants - in this case using the estuary as juveniles and usually showing preference for areas with lower salinity (Leitão *et al.* 2007). These groups showed higher biomass in winter and autumn, possibly reflecting the growth and production of the species after recruitment earlier in the year (Rosecchi & Crivelli 1992; Dinis *et al.* 1999; Gonçalves *et al.* 2003), as suggested by a higher species richness of group 4 observed in summer. Higher temperatures could also increase the number of omnivorous species (present in group 4) as shown in GLM, since it has been shown that omnivorous species benefit from drought conditions, where temperature is higher, because they have the capacity to adapt their diet from wet to dry periods, being therefore more tolerant to drought conditions than invertebrate feeders (Chessman 2013; Baptista *et al.* 2015b).

PERMANOVA revealed inter-annual differences in groups 5 and 6 for all functional group measures (biomass, density and species richness) which may be related to changes in climate patterns, especially regarding precipitation and runoff which varied greatly (Nyitrai *et al.* 2012). Consistently with our results, several drought and non-drought years were identified between 2003 to 2010 by Baptista *et al.* (2010) which also reported higher mean density of some species of our group 5 in non-drought years (namely *Gobius niger*, *Pomatoschistus minutus*, *Solea solea* and *Aphia minuta)* and of the majority of species in our group 6 (*Anguilla Anguilla*, *Dicentrarchus labrax*, *Platichthys flesus*, *Pomatoschistus microps* and *Liza ramada)*. Inter-annual differences in biomass of group 2 and 4, density of groups 1, 2 and 3 and number of species of group 1 could also be related to precipitation, since the highest values were registered for 2003, considered a regular hydrologic year in terms of precipitation by Nyitrai *et al.* (2012).

Generalized Linear Models revealed significant connections between salinity, temperature and oxygen and fish functional trait measures, which is consistent with other studies carried out in estuaries which observed effects of these environmental variables in the structure of estuarine assemblages (Marshall & Elliott 1998; Araújo *et al.* 1999; Akin *et al.* 2003). For example, França *et al.* (2011) reported that upstream-downstream temperature and salinity gradient within the Mondego estuary were significant predictors of species richness, although this was shown only for May and July 2006. In our study, the majority of traits were positively correlated with salinity and temperature and negatively for dissolved oxygen. However, relationships between traits and environmental conditions seem to be complex since, for example, density and species richness of groups 1, 2, 3 and 5 is higher at station M (at the mouth of the estuary) which presents higher dissolved oxygen, but oxygen showed a negative (but very weak, with 0.5 to 7.2% of variance explained by the GLM) correlation with most of the traits. This likely reflects a mixture of spatial, seasonal and inter-annual interactive effects of these environmental variables on the fish assemblage. The lower values of trait metrics for stations S1 and N2 can be related to lower salinities in N2, as observed by Leitão *et al.* (2007), and lower temperatures in S1. Also, there are inter-annual differences related to salinity, since in 2006, when lower values of most traits were registered, salinity decreased in the estuary, possibly because of higher precipitation (Nyitrai *et al.* 2012).

Mobility traits varied with temperature, which is probably related to increased occasional migrations of marine species and of juvenile life stages (Akin *et al.* 2003), with sedentary species responding positively to higher temperature and salinity, which is reinforced by the observed increase in density and species

richness of these species in spring and summer and an evident decrease in autumn. This was also observed for species with very small body size and freshwater species, which respond positively to temperature (but negatively to salinity, in the case of freshwater species). However, the predominant variations in trait measures were spatial and not seasonal, for all traits, which reflects differences in environmental variables between stations, as was also observed by Leitão *et al.* (2007) and Baptista *et al.* (2010). Additionally, hunters respond positively and strongly to salinity (and negatively to temperature), as well as species with a macrocarnivorous diet, because all species presenting these traits are marine species (better adapted to polyhaline and euhaline conditions) (Whitfield *et al.* 2012). Invertebrate feeders responded positively to temperature and salinity, constituting the majority of the fish assemblage in the Mondego (Nyitrai *et al.* 2012). These results are consistent with general patterns in temperate regions, where the abundance of benthic invertebrates decreases in autumn and winter and increases in summer, after spring recruitment, which is primarily driven by environmental conditions (Chainho *et al.* 2007). Thus, the positive response of invertebrate feeders to these variables may be related to food availability. This type of diet is very common in European estuaries, benefiting from the high productivity of estuarine systems (Franco *et al.* 2006; França *et al.* 2009).

In contrast with most traits, biomass and species richness of detritivores were negatively correlated with salinity, and their density was negatively correlated with temperature. The detritivorous feeders in the Mondego estuary fish assemblage (i.e. *Mugil cephalus* and *Liza ramada*) belong to group 6, which showed higher biomass and number of species for station N2, with lower salinities (Dolbeth *et al.* 2008). This could be explained by higher amount of detritus available in lower saline conditions, where decomposition of macroalgae occurs, producing detritus and inorganic nutrients (Martins *et al.* 1999; Whitfield *et al.* 2006). The general response of traits to oxygen was negative, but as oxygen varied between 62-120%, this result can hardly be attributed to excessive levels of dissolved oxygen, which might affect the rate of oxygen uptake by fish (Fry 1971; Kramer 1987). Still, filtering and territorial species, such as *Ammodytes tobianus*, showed positive response to oxygen due to its capacity to regulate oxygen uptake, lowering its metabolism (Behrens & Steffensen 2006) and improving filter feeding opportunity in agitated waters.

All traits showed temporal responses, revealing a strong effect of factor year in the functional composition of the fish assemblage despite a smaller seasonal effect. This latter result is different from other studies such as Shimadzu *et al.* (2013), which observed significant seasonal effects in an estuarine fish community, and Akin *et al.* (2003) which underlined the role of seasonally resident marine species in estuarine fish assemblages. 2003 was generally the year with higher values in traits for all measures, probably because it showed regular precipitation (Nyitrai *et al.* 2012) and high temperature (positively correlated to most traits) (Martinho *et al.* 2010). After 2004, anthropogenic activities (regulation by dams and deepening of the main navigation channel), together with the occurrence of drought events, led to higher incursion of seawater and lower freshwater runoff, thus increasing the salinity in the estuary and the number of marine species (Baptista *et al.* 2015b). In 2006, precipitation was considerably higher than in the other years, thus leading to a decrease in salinity in the estuary (Nyitrai *et al.* 2012), which probably explains the lower values in most traits for this year.

Functional redundancy of the Mondego estuary fish assemblage

Generally, estuarine systems are naturally faced with highly dynamic environmental conditions and their communities present high natural resilience to change, compared with other aquatic ecosystems, because organisms living in estuaries have the capacity of tolerating diverse environmental variations and adjust to stressful conditions, which increases the ability of estuaries to absorb disturbance (Elliott &

Whitfield 2011). Functional redundancy of the fish assemblage in the Mondego estuary showed seasonal and spatial variation, which is consistent with differences in functional composition previously discussed. Higher functional redundancy in station N2 (p-value < 0.05) indicates that a hypothetical loss of species in this station is likely to have a minor effect in ecosystem functioning (Ricotta et al. In press) and in the resilience of ecosystem services (Walker et al. 1999; Folke et al. 2004). The opposite could be expected for station S1 where the fish assemblage showed lower functional redundancy (p-value < 0.05). Seasonal variations in functional redundancy for density of species may be related to recruitment of juveniles of several marine species to the estuary (Vasconcelos et al. 2010), since spring (the main recruitment period) is the season with higher functional redundancy.

Looking at the annual functional redundancy within each functional group, functional group 7 showed null values, possibly being the group most vulnerable to disturbance, since it is composed by only three freshwater species which have been decreasing in the estuary (Leitão *et al.* 2007; Baptista *et al.* 2010; Dolbeth *et al.* 2013). Functional groups 1 and 4 showed lower values of functional redundancy comparing to the remaining groups, which might possibly mean the functions they perform would become more vulnerable to the loss of species (Gunderson 2000). Also, that would probably affect the ecosystem services that these groups can provide (nutrient cycling, biological control and coastal protection), possibly decreasing their resilience (Micheli & Halpern 2005; Muntadas *et al.* 2016). However, ecosystem functioning is a product of multiple variables (Murray *et al.* 2014) and as such, it results from a complex interaction of different components in the ecosystem, including distinct groups of organisms (besides fish), which should be considered when accounting the provision of ecosystem services. Functional groups 5 and 6 showed the highest functional redundancies, which may implicate the ecosystem services they are capable to provide are perhaps more likely to be maintained (Yachi & Loreau 1999; Fonseca & Ganade 2001; Ricotta *et al.* In press). Spatial variation in redundancy of groups 5 and 6 may be related with differences in their distribution between stations, with higher redundancies in stations M and N2 (for groups 5 and 6, respectively) where they also showed higher biomass. Furthermore, environmental variables may also be connected to these patterns, since salinity is negatively correlated to detritivores and species with tolerance to freshwater, which are both present in group 6, and N2 is the station with the lowest salinities. Seasonal variation in functional redundancy for groups 3 and 5 may be related with differences in temperature, with lower values of functional redundancy observed in summer, thus indicating higher functional diversity (de Bello *et al.* 2007). Temperature affects fish reproduction and therefore is related with recruitment dynamics, which is often related to seasonal changes in the composition of fish assemblages (Potter *et al.* 2001; Attrill & Power 2002; Akin *et al.* 2003; Vinagre *et al.* 2009; Nyitrai *et al.* 2013). This may explain why functional redundancy is higher in winter for group 3 and higher in autumn for group 5. Moreover, with higher temperatures, salinity tends to increase, which would allow marine species such as *Arnoglossus laterna*, *Buglossidium luteum* and *Pegusa lascaris* to enter the estuary, probably explaining the differences in functional redundancy for group 3.

Changes in precipitation could have influenced inter-annual variation of functional redundancy for density of group 6. Although group 6 is comprised by species that tolerate a wide range of salinities (marine, brackish and freshwater), it is also composed by estuarine residents (*Pomatoschistus microps*) and species that use the estuary as a nursery area (*Dicentrarchus labrax* and *Platichthys flesus*), which were affected by changes in precipitation and river runoff showing higher abundances in non-drought years i.e. with higher freshwater flow (Martinho *et al.* 2007; Martinho *et al.* 2009; Baptista *et al.* 2010). For density of group 2, inter-annual differences in functional redundancy may be explained by variations in salinity (Baptista *et al.* 2015b), since this group is comprised by marine species which varied inter-

annually in density. One of the most abundant species in the estuary, *Solea solea* (group 5) is marine and depends on the estuary as a sheltered habitat for juveniles. It showed higher densities and production in non-drought years (Dolbeth *et al.* 2008; Baptista *et al.* 2010) which could be an explanation for inter-annual differences in functional redundancy for biomass of group 5, since *S. solea* contributes significantly to biomass of this group. Furthermore, another species in group 5, *Pegusa lascaris,* only appeared in the estuary in 2005 (Martinho *et al.* 2007) which could have influenced the functional redundancy of this group (which was lower in 2005 than in 2004) by increasing species richness (Rosenfeld 2002) and also by affecting functional diversity, calculated with Rao's quadratic entropy (Rao 1982), which in turn is influenced by abundance of species (Botta-Dukát 2005; de Bello *et al.* 2007).

Assessing functional redundancy in the Mondego estuary is important for conservation, since a lower variation in ecosystem functioning is expected in more functionally redundant communities (Naeem 1998), which means that the loss of species is less likely to impact ecosystem functions in these situations (Sasaki *et al.* 2009; Mouillot *et al.* 2013). Additionally, as functional redundancy has been related to ecosystem resilience, it could be an important tool to predict changes in functions under threatening conditions (Walker 1992; Yachi & Loreau 1999; Lavorel & Garnier 2002; Muntadas *et al.* 2016). However, this depends on the vulnerability of species to disturbance (Hughes *et al.* 2005) and also on their interactions with the environment, which influence their functional role (Wellnitz & Poff 2001; Murray *et al.* 2014). Additionally, rare species can contribute significantly to maintain ecosystem functioning (Mouillot *et al.* 2013), because they may respond differently to stress (Boero 1994; Walker *et al.* 1999) and thus may have the potential to sustain important ecosystem functions under changing environmental conditions (Flöder *et al.* 2010). Moreover, they increase functional diversity (Richardson *et al.* 2012) and may be able to perform functions complementary to those delivered by common species, as a result of their different functional attributes (Lavergne *et al.* 2003; Mouillot *et al.* 2013). This seems to be for example the case of *Sparus aurata*, the only omnivorous species in group 1 and the only possible contributor to the service of coastal protection and disturbance prevention in this group (three of the remaining species are macrocarnivorous and one is an invertebrate feeder). Such species assume a key functional role. Although the Mondego estuary is not the most threatened in the Portuguese coast, it is constantly under anthropogenic pressure, therefore it is important to consider the effects of human activities on biodiversity of this transitional system, which can have significant impacts in ecosystem functioning and thus in the provision of ecosystem services (Elmqvist *et al.* 2003; Pinto *et al.* 2014). Finally, knowledge of the functional redundancy of each functional group is important to understand its resilience to disturbance and its influence in ecosystem functioning and services (Fonseca & Ganade 2001; Bellwood *et al.* 2004; Mouillot *et al.* 2013). Higher values of annual functional redundancy were observed for groups 5 and 6, suggesting that these groups are potentially more likely to preserve the same functions, even with loss of species following disturbance (Walker *et al.* 1999; Folke *et al.* 2004). Also, these groups are the most represented in the fish assemblage (in terms of biomass, density and number of species), which reinforces their resilience. Group 5 is unique because it contains filterer and planktivorous species, which may influence the provision of ecosystem services such as waste treatment and assimilation (Zhou *et al.* 1999) and climate regulation (Schindler *et al.* 1997). Therefore, these services are possibly the more resilient to disturbance. On the other hand, the lower annual functional redundancy observed for groups 1 and 4 may implicate they are more vulnerable to species loss (Mouillot *et al.* 2014), which may decrease the resilience of the ecosystem services they can provide (nutrient cycling, biological control and coastal protection and disturbance prevention).

Functional redundancy must be taken into account, as it may allow for some species in an ecosystem to compensate for the loss of others, maintaining important functions in the estuary (Biggs *et al.* 2012). There are, however, other ways to quantify functional redundancy, for example, van der Linden *et al.* (2016) divides functional diversity (quantified as Rao's quadratic entropy) for species diversity (quantified as Simpson's diversity index), explaining that functional redundancy measures the amount of trait similarity between species in a community. There is still a need for satisfactory methods to quantify functional redundancy, according to Carmona *et al.* (2016), despite its recognized importance for resilience.

Conclusions

Considering the evidence of connections between traits and ecosystem functions, and also the wide variety of traits in fishes (Villéger *et al.* 2012), there is great potential in exploring these links for human well-being. This study is the first one to assess the provision of ecosystem services by the fish assemblage of the Mondego estuary and estimate their resilience, which is an important step towards ecosystem-based management and conservation efforts (Pinto *et al.* 2014). Also, it constitutes a development of knowledge about the role of fish in the performance of important functions within the estuary, which is affected by fisheries, similarly to other estuaries worldwide (Blaber 2000). Nevertheless, it is important to consider the limitations of this study, especially concerning the subjectivity of the functional groups approach (Murray *et al.* 2014) and the uncertainty about calculation of functional redundancy (Rosenfeld 2002). Besides, other types of services could be studied within this estuary, such as provisioning and habitat services. Overall, there is a long way ahead in ecosystem service assessment in estuarine systems in general, but this study highlighted the importance of fishes in regulating ecosystem services and the need for more functional composition studies, especially investigating the contribution of fish and other groups of organisms in estuaries towards the delivery of ecosystem services.

Literature cited

Abecasis D., Bentes L. & Erzini K. (2009). Home range, residency and movements of Diplodus sargus and Diplodus vulgaris in a coastal lagoon: Connectivity between nursery and adult habitats. *Estuarine, Coastal and Shelf Science*, 85: 525-529.

Adámek Z. & Maršálek B. (2013). Bioturbation of sediments by benthic macroinvertebrates and fish and its implication for pond ecosystems: a review. *Aquaculture International*, 21: 1-17.

Akin S., Winemiller K.O. & Gelwick F.P. (2003). Seasonal and spatial variations in fish and macrocrustacean assemblage structure in Mad Island Marsh estuary, Texas. *Estuarine, Coastal and Shelf Science*, 57: 269-282.

Alves A.S., Caetano A., Costa J.L., Costa M.J. & Marques J.C. (2015). Estuarine intertidal meiofauna and nematode communities as indicator of ecosystem's recovery following mitigation measures. *Ecological Indicators*, 54: 184-196.

Anderson M.J., Gorley R.N. & Clarke K.R. (2008). *PERMANOVA + for PRIMER: Guide to Software and Statistical Methods* PRIMER-E Plymouth, UK.

Andersson G., Berggren H., Cronberg G. & Gelin C. (1978). Effects of planktivorous and benthivorous fish on organisms and water chemistry in eutrophic lakes. *Hydrobiologia*, 59: 9-15.

Araújo F.G., Bailey R.G. & Williams W.P. (1999). Spatial and temporal variations in fish populations in the upper Thames estuary. *Journal of Fish Biology*, 55: 836-853.

Assessment M.E. (2005). *Ecosystems and human well-being: synthesis*. Island Press, Washington D. C.

Attrill M.J. & Power M. (2002). Climatic influence on a marine fish assemblage. *Nature*, 417: 275-278.

Auster P.J. & Link J.S. (2009). Compensation and recovery of feeding guilds in a northwest Atlantic shelf fish community. *Marine Ecology Progress Series*, 382: 163-172.

Avnimelech Y., Kochva M. & Hargreaves J.A. (1999). Sedimentation and resuspension in earthen fish ponds. *Journal of the World Aquaculture Society*, 30: 401-409.

Baptista J., Martinho F., Dolbeth M., Viegas I., Cabral H. & Pardal M. (2010). Effects of freshwater flow on the fish assemblage of the Mondego estuary (Portugal): comparison between drought and non-drought years. *Marine and Freshwater Research*, 61: 490-501.

Baptista J., Martinho F., Nyitrai D., Pardal M.A. & Dolbeth M. (2015a). Long-term functional changes in an estuarine fish assemblage. *Mar Pollut Bull*, 97: 125-134.

Baptista J., Martinho F., Nyitrai D., Pardal M.A. & Dolbeth M. (2015b). Long-term functional changes in an estuarine fish assemblage. *Marine Pollution Bulletin*, 97: 125-134.

Baptista J., Pato P., Pereira E., Duarte A.C. & Pardal M.A. (2013). PCBs in the fish assemblage of a southern European estuary. *Journal of Sea Research*, 76: 22-30.

Barbier E.B., Hacker S.D., Kennedy C., Koch E.W., Stier A.C. & Silliman B.R. (2011). The value of estuarine and coastal ecosystem services. *Ecological Monographs*, 81: 169-193.

Beaumont N.J., Austen M.C., Atkins J.P., Burdon D., Degraer S., Dentinho T.P., Derous S., Holm P., Horton T., van Ierland E., Marboe A.H., Starkey D.J., Townsend M. & Zarzycki T. (2007). Identification, definition and quantification of goods and services provided by marine biodiversity: implications for the ecosystem approach. *Marine Pollution Bulletin*, 54: 253-265.

Behrens J.W. & Steffensen J.F. (2006). The effect of hypoxia on behavioural and physiological aspects of lesser sandeel, Ammodytes tobianus (Linnaeus, 1785). *Marine Biology*, 150: 1365-1377.

Bellwood D.R., Hoey A.S. & Choat J.H. (2003). Limited functional redundancy in high diversity systems: resilience and ecosystem function on coral reefs. *Ecology Letters*, 6: 281-285.

Bellwood D.R., Hughes T.P., Folke C. & Nystrom M. (2004). Confronting the coral reef crisis. *Nature*, 429: 827-833.

Biggs R., Schlüter M., Biggs D., Bohensky E.L., BurnSilver S., Cundill G., Dakos V., Daw T.M., Evans L.S., Kotschy K., Leitch A.M., Meek C., Quinlan A., Raudsepp-Hearne C., Robards M.D., Schoon M.L., Schultz L. & West P.C. (2012). Toward principles for enhancing the resilience of ecosystem services. *Annual Review of Environment and Resources*, 37: 421-448.

Bilby R.E., Fransen B.R. & Bisson P.A. (1996). Incorporation of nitrogen and carbon from spawning coho salmon into the trophic system of small streams: evidence from stable isotopes. *Canadian Journal of Fisheries and Aquatic Sciences*, 53: 164-173.

Blaber S. (2000). Effects of fishing on the structure and functioning of estuarine and nearshore ecosystems. *ICES Journal of Marine Science*, 57: 590-602.

Boero F. (1994). Fluctuations and variations in coastal marine environments. *Marine Ecology-Pubblicazioni Della Stazione Zoologica Di Napoli I*, 15: 3-25.

Böhnke-Henrichs A., Baulcomb C., Koss R., Hussain S.S. & de Groot R.S. (2013). Typology and indicators of ecosystem services for marine spatial planning and management. *Journal of Environmental Management*, 130: 135-145.

Borja A., Dauer D.M., Elliott M. & Simenstad C.A. (2010). Medium- and long-term recovery of estuarine and coastal ecosystems: patterns, rates and restoration effectiveness. *Estuaries and Coasts*, 33: 1249-1260.

Botta-Dukát Z. (2005). Rao's quadratic entropy as a measure of functional diversity based on multiple traits. *Journal of Vegetation Science*, 16: 533-540.

Boyd J. & Banzhaf S. (2007). What are ecosystem services? The need for standardized environmental accounting units. *Ecological Economics*, 63: 616-626.

Brawley S.H. & Adey W.H. (1977). Territorial behavior of threespot damselfish (Eupomacentrus planifrons) increases reef algal biomass and productivity. *Environmental Biology of Fishes*, 2: 45-51.

Breheny P. & Burchett W. (2016). Visualization of regression models. *R package version 2.2-2*.

Bremner J., Rogers S.I. & Frid C.L.J. (2003). Assessing functional diversity in marine benthic ecosystems: a comparison of approaches. *Marine Ecology Progress Series*, 254: 11-25.

Burkepile D.E. & Hay M.E. (2008). Herbivore species richness and feeding complementarity affect community structure and function on a coral reef. *Proceedings of the National Academy of Sciences of the United States of America*, 105: 16201-16206.

Cabral H. & Costa M.J. (1999). Differential use of nursery areas within the Tagus estuary by sympatric soles, Solea solea and Solea senegalensis. *Environmental Biology of Fishes*, 56: 389-397.

Caddy J.F. & Garibaldi L. (2000). Apparent changes in the trophic composition of world marine harvests: the perspective from the FAO capture database. *Ocean & Coastal Management*, 43: 615-655.

Cardinale B.J., Duffy J.E., Gonzalez A., Hooper D.U., Perrings C., Venail P., Narwani A., Mace G.M., Tilman D., Wardle D.A., Kinzig A.P., Daily G.C., Loreau M., Grace J.B., Larigauderie A., Srivastava D.S. & Naeem S. (2012). Biodiversity loss and its impact on humanity. *Nature*, 486: 59-67.

Cardoso P.G., Pardal M.A., Lillebø A.I., Ferreira S.M., Raffaelli D. & Marques J.C. (2004). Dynamic changes in seagrass assemblages under eutrophication and implications for recovery. *Journal of Experimental Marine Biology and Ecology*, 302: 233-248.

Carmona C.P., de Bello F., Mason N.W. & Leps J. (2016). Traits Without Borders: Integrating Functional Diversity Across Scales. *Trends in Ecology and Evolution*, 31: 382-394.

Carpenter S.R., Cottingham K.L. & Schindler D.E. (1992). Biotic feedbacks in lake phosphorus cycles. *Trends in Ecology and Evolution*, 7: 332-336.

Carpenter S.R., Kitchell J.F. & Hodgson J.R. (1985). Cascading trophic interactions and lake productivity. *BioScience*, 35: 634-639.

Carpenter S.R., Mooney H.A., Agard J., Capistrano D., Defries R.S., Diaz S., Dietz T., Duraiappah A.K., Oteng-Yeboah A., Pereira H.M., Perrings C., Reid W.V., Sarukhan J., Scholes R.J. & Whyte A. (2009). Science for managing ecosystem services: Beyond the Millennium Ecosystem Assessment. *Proceedings of the National Academy of Sciences of the United States of America*, 106: 1305-1312.

Chainho P., Costa J.L., Chaves M.L., Dauer D.M. & Costa M.J. (2007). Influence of seasonal variability in benthic invertebrate community structure on the use of biotic indices to assess the ecological status of a Portuguese estuary. *Marine Pollution Bulletin*, 54: 1586-1597.

Cheal A.J., MacNeil M.A., Cripps E., Emslie M.J., Jonker M., Schaffelke B. & Sweatman H. (2010). Coral-macroalgal phase shifts or reef resilience: links with diversity and functional roles of herbivorous fishes on the Great Barrier Reef. *Coral Reefs*, 29: 1005-1015.

Chessman B.C. (2013). Identifying species at risk from climate change: Traits predict the drought vulnerability of freshwater fishes. *Biological Conservation*, 160: 40-49.

Clarke K.R. & Gorley R.N. (2006). *PRIMER v6: User manual/tutorial.* PRIMER-E, Plymouth UK.

Cole A.J., Pratchett M.S. & Jones G. (2008). Diversity and functional importance of coral-feeding fishes on tropical coral reefs. *Fish and Fisheries*, 9: 286-307.

Costanza R., dArge R., deGroot R., Farber S., Grasso M., Hannon B., Limburg K., Naeem S., ONeill R.V., Paruelo J., Raskin R.G., Sutton P. & vandenBelt M. (1997). The value of the world's ecosystem services and natural capital. *Nature*, 387: 253-260.

Costello M.J., Claus S., Dekeyzer S., Vandepitte L., Tuama E.O., Lear D. & Tyler-Walters H. (2015). Biological and ecological traits of marine species. *PeerJ*, 3: 1-29.

Cury P., Shannon L. & Shin Y.J. (2003). The functioning of marine ecosystems: a fisheries perspective. *Responsible Fisheries in the Marine Ecosystem*: 103-123.

D'Agata S., Mouillot D., Kulbicki M., Andrefouet S., Bellwood D.R., Cinner J.E., Cowman P.F., Kronen M., Pinca S. & Vigliola L. (2014). Human-mediated loss of phylogenetic and functional diversity in coral reef fishes. *Current Biology*, 24: 555-560.

Daily G.C. (1997). *Nature's services: societal dependence on natural ecosystems*, Washington.

de Bello F., Lavorel S., Díaz S., Harrington R., Cornelissen J.H.C., Bardgett R.D., Berg M.P., Cipriotti P., Feld C.K., Hering D., Martins da Silva P., Potts S.G., Sandin L., Sousa J.P., Storkey J., Wardle D.A. & Harrison P.A. (2010). Towards an assessment of multiple ecosystem processes and services via functional traits. *Biodiversity and Conservation*, 19: 2873-2893.

de Bello F., Leps J., Lavorel S. & Moretti M. (2007). Importance of species abundance for assessment of trait composition: an example based on pollinator communities. *Community Ecology*, 8: 163-170.

de Groot R., Fisher B. & Christie M. (2010). Integrating the ecological and economic dimensions in biodiversity and ecosystem service valuation. In: *The Economics of Ecosystems and Biodiversity: The Ecological and Economic Foundations* (ed. Kadekodi GK), pp. 2 - 40.

Debastiani V.J. & Pillar V.D. (2012). SYNCSA — R tool for analysis of metacommunities based on functional traits and phylogeny of the community components. *Bioinformatics*, 28: 2067-2068.

Deegan L.A. (1993). Nutrient and energy transport between estuaries and coastal marine ecosystems by fish migration. *Canadian Journal of Fisheries and Aquatic Sciences*, 50: 75-79.

Díaz S., Fargione J., Chapin F.S., 3rd & Tilman D. (2006). Biodiversity loss threatens human well-being. *PLoS Biology*, 4: 1300-1305.

Díaz S., Lavorel S., de Bello F., Quetier F., Grigulis K. & Robson T.M. (2007). Incorporating plant functional diversity effects in ecosystem service assessments. *Proceedings of the National Academy of Sciences of the United States of America*, 104: 20684-20689.

Díaz S., Quetier F., Caceres D.M., Trainor S.F., Perez-Harguindeguy N., Bret-Harte M.S., Finegan B., Pena-Claros M. & Poorter L. (2011). Linking functional diversity and social actor strategies in a framework for interdisciplinary analysis of nature's benefits to society. *Proceedings of the National Academy of Sciences of the United States of America*, 108: 895-902.

Dinis M.T., Ribeiro L., Soares F. & Sarasquete C. (1999). A review on the cultivation potential of Solea senegalensis in Spain and in Portugal. *Aquaculture*, 176: 27-38.

Dolbeth M., Cardoso P., Grilo T., Raffaelli D. & Pardal M.A. (2013). Drivers of estuarine benthic species distribution patterns following a restoration of a seagrass bed: a functional trait analyses. *Marine Pollution Bulletin*, 72: 47-54.

Dolbeth M., Cardoso P.G., Grilo T.F., Bordalo M.D., Raffaelli D. & Pardal M.A. (2011). Long-term changes in the production by estuarine macrobenthos affected by multiple stressors. *Estuarine Coastal and Shelf Science*, 92: 10-18.

Dolbeth M., Dolédec S. & Pardal M. (2015). Relationship between functional diversity and benthic secondary production in a disturbed estuary. *Marine Ecology Progress Series*, 539: 33-46.

Dolbeth M., Martinho F., Viegas I., Cabral H. & Pardal M.A. (2008). Estuarine production of resident and nursery fish species: Conditioning by drought events? *Estuarine, Coastal and Shelf Science*, 78: 51-60.

Done T.J., Dayton P.K., Dayton A.E. & Steger R. (1991). Regional and local variability in recovery of shallow coral communities: Moorea, French Polynesia and central Great Barrier Reef. *Coral Reefs*, 9: 183-192.

Elliott M. & Whitfield A.K. (2011). Challenging paradigms in estuarine ecology and management. *Estuarine, Coastal and Shelf Science*, 94: 306-314.

Elliott M., Whitfield A.K., Potter I.C., Blaber J.M., Cyrus D.P., Nordlie F.G. & Harrison T.D. (2007). The guild approach to categorizing estuarine fish assemblages: a global review. *Fish and Fisheries*, 8: 241-268.

Elmqvist T., Folke C., Nyström M., Peterson G., Bengtsson J., Walker B. & Norberg J. (2003). Response diversity, ecosystem change, and resilience. *Frontiers in Ecology and the Environment*, 1: 488-494.

Fisher B., Turner R.K. & Morling P. (2009). Defining and classifying ecosystem services for decision making. *Ecological Economics*, 68: 643-653.

Flecker A.S. (1992). Fish trophic guilds and the structure of a tropical strea: weak direct vs. strong indirect effects. *Ecology*, 73: 927-940.

Flecker A.S., Taylor B.W., Bernhardt E.S., Hood J.M., Cornwell W.K., Cassatt S.R., Vanni M.J. & Altman N.S. (2002). Interactions between herbivorous fishes and limiting nutrients in a tropical stream ecosystem. *Ecology*, 83: 1831-1844.

Flöder S., Jaschinski S., Wells G. & Burns C.W. (2010). Dominance and compensatory growth in phytoplankton communities under salinity stress. *Journal of Experimental Marine Biology and Ecology*, 395: 223-231.

Folke C., Carpenter S., Walker B., Scheffer M., Elmqvist T., Gunderson L. & Holling C.S. (2004). Regime shifts, resilience, and biodiversity in ecosystem management. *Annual Review of Ecology, Evolution, and Systematics*, 35: 557-581.

Fonseca C.R. & Ganade G. (2001). Species functional redundancy, random extinctions and the stability of ecosystems. *Journal of Ecology*, 89: 118-125.

França S., Costa M.J. & Cabral H.N. (2009). Assessing habitat specific fish assemblages in estuaries along the Portuguese coast. *Estuarine, Coastal and Shelf Science*, 83: 1-12.

França S., Costa M.J. & Cabral H.N. (2011). Inter- and intra-estuarine fish assemblage variability patterns along the Portuguese coast. *Estuarine, Coastal and Shelf Science*, 91: 262-271.

Franco A., Franzoi P., Malavasi S., Riccato F., Torricelli P. & Mainardi D. (2006). Use of shallow water habitats by fish assemblages in a Mediterranean coastal lagoon. *Estuarine, Coastal and Shelf Science*, 66: 67-83.

Fry F.E.J. (1971). The effect of environmental factors on the physiology of fish. In: *Fish Physiology* (eds. Hoar WS & Randall DJ). Academic Press New York, pp. 1-98.

García-Llorente M., Martín-López B., Díaz S. & Montes C. (2011). Can ecosystem properties be fully translated into service values? An economic valuation of aquatic plant services. *Ecological Applications*, 21: 3083 - 3103.

Garcia A., Vieira J.P. & Winemiller K.O. (2001). Dynamics of the shallow-water fish assemblage of the Patos Lagoon estuary (Brazil) during cold and warm ENSO episodes. *Journal of Fish Biology*, 59: 1218-1238.

Gelwick F.P., Stock M.S. & Matthews W.J. (1997). Effects of fish, water depth, and predation risk on patch dynamics in a north-temperate river ecosystem. *Oikos*, 80: 382-398.

Genner M.J., Sims D.W., Southward A.J., Budd G.C., Masterson P., McHugh M., Rendle P., Southall E.J., Wearmouth V.J. & Hawkins S.J. (2010). Body size-dependent responses of a marine fish assemblage to climate change and fishing over a century-long scale. *Global Change Biology*, 16: 517-527.

Gerino M., Stora G., François-Carcaillet F., Gilbert F., Poggiale J.-C., Mermillod-Blondin F., Desrosiers G. & Vervier P. (2003). Macro-invertebrates functional groups in freshwater and marine sediments: a commun mechanistic classification. *Vie et Milieu*: 221-231.

Gonçalves J.M.S., Bentes L., Coelho R., Correia C., Lino P.G., Monteiro C.C., Ribeiro J. & Erzini K. (2003). Age and growth, maturity, mortality and yield-per-recruit for two banded bream (Diplodus vulgaris Geoffr.) from the south coast of Portugal. *Fisheries Research*, 62: 349-359.

Gower J.C. (1966). Some Distance Properties of Latent Root and Vector Methods Used in Multivariate Analysis. *Biometrika*, 53: 325-338.

Grömping U. (2006). Relative importance for linear regression in R: The package relaimpo. *Journal of Statistical Software*, 17: 1-27.

Gunderson L. (2000). Ecological resilience - in theory and application. *Annual Review of Ecology, Evolution, and Systematics*: 425-439.

Hall R.O., Jr., Koch B.J., Marshall M.C., Taylor B.W. & Tronstad L.M. (2007). How body size mediates the role of animals in nutrient cycling in aquatic ecosystems. In: *Body size and the structure and function of aquatic ecosystems* (eds. Hildrew AG, Raffaelli DG & Edmonds-Brown R). Cambridge University Press New York.

Han T., Zhang H., Hu W., Deng J., Li Q. & Zhu G. (2015). Research on self-purification capacity of Lake Taihu. *Environmental Science and Pollution Research*, 22: 8201-8215.

Harrison P.A., Berry P.M., Simpson G., Haslett J.R., Blicharska M., Bucur M., Dunford R., Egoh B., Garcia-Llorente M., Geamana N., Geertsema W., Lommelen E., Meiresonne L. & Turkelboom F. (2014). Linkages between biodiversity attributes and ecosystem services: A systematic review. *Ecosystem Services*, 9: 191-203.

Hattam C., Atkins J.P., Beaumont N., Borger T., Bohnke-Henrichs A., Burdon D., de Groot R., Hoefnagel E., Nunes P.A.L.D., Piwowarczyk J., Sastre S. & Austen M.C. (2015). Marine ecosystem services: Linking indicators to their classification. *Ecological Indicators*, 49: 61-75.

Heck K.L., Pennock J.R., Valentine J.F., Coen L.D. & Sklenar S.A. (2000). Effects of nutrient enrichment and small predator density on seagrass ecosystems: An experimental assessment. *Limnology and Oceanography*, 45: 1041-1057.

Heck K.L. & Valentine J.F. (2006). Plant–herbivore interactions in seagrass meadows. *Journal of Experimental Marine Biology and Ecology*, 330: 420-436.

Henriques S., Pais M.P., Batista M.I., Costa M.J. & Cabral H.N. (2013). Response of fish-based metrics to anthropogenic pressures in temperate rocky reefs. *Ecological Indicators*, 25: 65-76.

Hixon M. & Brostoff W. (1996). Sucession and herbivory: effects of differential fish grazing on hawaiian coral-reef algae. *Ecological Monographs*, 66: 67-90.

Hoey A.S. & Bellwood D.R. (2011). Suppression of herbivory by macroalgal density: a critical feedback on coral reefs? *Ecology Letters*, 14: 267-273.

Holling C.S. (1973). Resilience and stability of ecological systems. *Annual Review of Ecology, Evolution, and Systematics*, 4: 1-23.

Holmlund C.M. & Hammer M. (1999). Ecosystem services generated by fish populations. *Ecological Economics*, 29: 253-268.

Hooper D.U., Chapin F.S., Ewel J.J., Hector A., Inchausti P., Lavorel S., Lawton J.H., Lodge D.M., Loreau M., Naeem S., Schmid B., Setala H., Symstad A.J., Vandermeer J. & Wardle D.A. (2005). Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs*, 75: 3-35.

Hoy J.B., Kauffman E.E. & O'Berg A.G. (1972). A large-scale field test og Gambusia affinis and chlorpyrifos for mosquito control. *Mosquito News*, 32: 161-171.

Hughes T.P., Bellwood D.R., Folke C., Steneck R.S. & Wilson J. (2005). New paradigms for supporting the resilience of marine ecosystems. *Trends in Ecology and Evolution*, 20: 380-386.

Iguchi K. & Yodo T. (2004). Impact of indigenous egg eaters on the early survival of exotic smallmouth bass. *Ecological Research*, 19: 469-474.

Katz T., Herut B., Genin A. & Angel D.L. (2002). Gray mullets ameliorate organically enriched sediments below a fish farm in the oligotrophic Gulf of Aqaba (Red Sea). *Marine Ecology Progress Series*, 234: 205-214.

Kennish M.J. (2002). Environmental threats and environmental future of estuaries. *Environmental Conservation*, 29: 78-107.

Koutsikopoulos C., Desaunay Y., Dorel D. & Marchand J. (1989). The role of coastal areas in the life history of sole (Solea solea L.) in the Bay of Biscay. *Scientia Marina*, 53: 567-575.

Kramer D.L. (1987). Dissolved oxygen and fish behavior. *Environmental Biology of Fishes*, 18: 81-92.

Kremen C. (2005). Managing ecosystem services: what do we need to know about their ecology? *Ecology Letters*, 8: 468-479.

Laliberté E., Wells J.A., Declerck F., Metcalfe D.J., Catterall C.P., Queiroz C., Aubin I., Bonser S.P., Ding Y., Fraterrigo J.M., McNamara S., Morgan J.W., Merlos D.S., Vesk P.A. & Mayfield M.M. (2010). Land-use intensification reduces functional redundancy and response diversity in plant communities. *Ecology Letters*, 13: 76-86.

Lavergne S., Garnier E. & Debussche M. (2003). Do rock endemic and widespread plant species differ under the Leaf–Height–Seed plant ecology strategy scheme? *Ecology Letters*, 6: 398-404.

Lavorel S. & Garnier E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology*, 16: 545-556.

Lavorel S. & Grigulis K. (2012). How fundamental plant functional trait relationships scale-up to trade-offs and synergies in ecosystem services. *Journal of Ecology*, 10.1111/j.1365-2745.2011.01914.x.

Leitão R., Martinho F., Cabral H.N., Neto J.M., Jorge I. & Pardal M.A. (2007). The fish assemblage of the Mondego estuary: composition, structure and trends over the past two decades. *Hydrobiologia*, 587: 269-279.

Liquete C., Piroddi C., Drakou E.G., Gurney L., Katsanevakis S., Charef A. & Egoh B. (2013). Current status and future prospects for the assessment of marine and coastal ecosystem services: a systematic review. *PLoS One*, 8: 1-15.

Luck G.W., Harrington R., Harrison P.A., Kremen C., Berry P.M., Bugter R., Dawson T.P., de Bello F., Diaz S., Feld C.K., Haslett J.R., Hering D., Kontogianni A., Lavorel S., Rounsevell M., Samways M.J., Sandin L., Settele J., Sykes M.T., van den Hove S., Vandewalle M. & Zobel M. (2009). Quantifying the Contribution of Organisms to the Provision of Ecosystem Services. *BioScience*, 59: 223-235.

Lyons K.G., Brigham C.A., Traut B.H. & Schwartz M.W. (2005). Rare species and ecosystem functioning. *Conservation Biology*, 19: 1019-1024.

Mackenzie B.R., Almesjo L. & Hansson S. (2004). Fish, fishing, and pollutant reduction in the Baltic Sea. *Environ Sci Technol*, 38: 1970-1976.

Maechler M., Rousseeuw P., Struyf A., Hubert M. & Hornik K. (2015). Cluster: Cluster Analysis Basics and Extensions. *R package version 2.0.3*.

Marshall S. & Elliott M. (1998). Environmental Influences on the fish assemblage of the Humber Estuary, U.K. *Estuarine, Coastal and Shelf Science*, 46: 175-184.

Martinho F., Dolbeth M., Viegas I., Baptista J., Cabral H.N. & Pardal M.A. (2010). Does the flatfish community of the Mondego estuary (Portugal) reflect environmental changes? *Journal of Applied Ichthyology*, 26: 843-852.

Martinho F., Dolbeth M., Viegas I., Teixeira C.M., Cabral H.N. & Pardal M.A. (2009). Environmental effects on the recruitment variability of nursery species. *Estuarine, Coastal and Shelf Science*, 83: 460-468.

Martinho F., Leitão R., Viegas I., Dolbeth M., Neto J.M., Cabral H.N. & Pardal M.A. (2007). The influence of an extreme drought event in the fish community of a southern Europe temperate estuary. *Estuarine, Coastal and Shelf Science*, 75: 537-546.

Martins I., Oliveira J.M., Flindt M.R. & Marques J.C. (1999). The effect of salinity on the growth rate of the macroalgae Enteromorpha intestinalis (Chlorophyta) in the Mondego estuary (west Portugal). *Acta Oecologica*, 20: 259-265.

Micheli F. & Halpern B.S. (2005). Low functional redundancy in coastal marine assemblages. *Ecology Letters*, 8: 391-400.

Mouillot D., Bellwood D.R., Baraloto C., Chave J., Galzin R., Harmelin-Vivien M., Kulbicki M., Lavergne S., Lavorel S., Mouquet N., Paine C.E., Renaud J. & Thuiller W. (2013). Rare species support vulnerable functions in high-diversity ecosystems. *PLoS Biology*, 11: 1-11.

Mouillot D., Graham N.A., Villeger S., Mason N.W. & Bellwood D.R. (2012). A functional approach reveals community responses to disturbances. *Trends in Ecology and Evolution*, 28: 167-177.

Mouillot D., Villeger S., Parravicini V., Kulbicki M., Arias-Gonzalez J.E., Bender M., Chabanet P., Floeter S.R., Friedlander A., Vigliola L. & Bellwood D.R. (2014). Functional over-redundancy and high functional vulnerability in global fish faunas on tropical reefs. *Proceedings of the National Academy of Sciences of the United States of America*, 111: 13757-13762.

Mumby P.J., Harborne A.R. & Brumbaugh D.R. (2011). Grouper as a Natural Biocontrol of Invasive Lionfish. *PLoS One*, 6.

Muntadas A., de Juan S. & Demestre M. (2016). Assessing functional redundancy in chronically trawled benthic communities. *Ecological Indicators*, 61: 882-892.

Murray F., Douglas A. & Solan M. (2014). Species that share traits do not necessarily form distinct and universally applicable functional effect groups. *Marine Ecology Progress Series*, 516: 23-34.

Naeem S. (1998). Species redundancy and ecosystem reliability. *Conservation Biology*, 12: 39-45.

Nally R.M. (1996). Hierarchical partitioning as an interpretative tool in multivariate inference. *Australian Journal of Ecology*, 21: 224-228.

Neto J.M., Teixeira H., Patrício J., Baeta A., Veríssimo H., Pinto R. & Marques J.C. (2010). The Response of Estuarine Macrobenthic Communities to Natural- and Human-Induced Changes: Dynamics and Ecological Quality. *Estuaries and Coasts*, 33: 1327-1339.

Nyitrai D., Martinho F., Dolbeth M., Baptista J. & Pardal M.A. (2012). Trends in estuarine fish assemblages facing different environmental conditions: combining diversity with functional attributes. *Aquatic Ecology*, 46: 201-214.

Nyitrai D., Martinho F., Dolbeth M., Rito J. & Pardal M.A. (2013). Effects of local and large-scale climate patterns on estuarine resident fishes: The example of Pomatoschistus microps and Pomatoschistus minutus. *Estuarine, Coastal and Shelf Science*, 135: 260-268.

Nyström M., Folke C. & Moberg F. (2000). Coral reef disturbance and resilience in a human-dominated environment. *Trends in Ecology and Evolution*, 15: 413-417.

Ordiano-Flores A., Galvan-Magana F. & Rosiles-Martinez R. (2011). Bioaccumulation of mercury in muscle tissue of yellowfin tuna, Thunnus albacares, of the eastern Pacific Ocean. *Biol Trace Elem Res*, 144: 606-620.

Pinto R., de Jonge V.N. & Marques J.C. (2014). Linking biodiversity indicators, ecosystem functioning, provision of services and human well-being in estuarine systems: application of a conceptual framework. *Ecological Indicators*, 36: 644-655.

Potter I.C., Bird D.J., Claridge P.N., Clarke K.R., Hyndes G.A. & Newton L.C. (2001). Fish fauna of the Severn Estuary. Are there long-term changes in abundance and species composition and are the recruitment patterns of the main marine species correlated? *Journal of Experimental Marine Biology and Ecology*, 258: 15-37.

Pratchett M.S., Hoey A.S. & Wilson S.K. (2014). Reef degradation and the loss of critical ecosystem goods and services provided by coral reef fishes. *Current Opinion in Environmental Sustainability*, 7: 37-43.

Qin J. & Threlkeld S. (1990). Experiental coparison of the effects of benthivorous fish and planktivorous fish on plankton community structure *Archiv für Hydrobiologie*, 119: 121-141.

Rao R.C. (1982). Diversity and dissimilarity coefficients: a unified approach. *Theroretical Population Biology*, 21: 24-43.

Raymundo L.J., Halford A.R., Maypa A.P. & Kerr A.M. (2009). Functionally diverse reef-fish communities ameliorate coral disease. *Proceedings of the National Academy of Sciences of the United States of America*, 106: 17067-17070.

Richardson S.J., Williams P.A., Mason N.W., Buxton R.P., Courtney S.P., Rance B.D., Clarkson B.R., Hoare R.J.B., St. John M.G. & Wiser S.K. (2012). Rare species drive local trait diversity in two geographically disjunct examples of a naturally rare alpine ecosystem in New Zealand. *Journal of Vegetation Science*, 23: 626-639.

Ricotta C., de Bello F., Moretti M., Caccianiga M., Cerabolini B.E.L. & Pavoine S. (In press). Measuring the functional redundancy of biological communities: A quantitative guide. *Methods in Ecology and Evolution*.

Roberts D.L. (2016). Labdsv: Ordination and Multivariate Analysis for Ecology. *R package version 1.8.0*.

Rodrigues E.T. & Pardal M.Â. (2015). Primary productivity temporal fluctuations in a nutrient-rich estuary due to climate-driven events. *Estuaries and Coasts*, 38: 1-12.

Rosecchi E. & Crivelli A.J. (1992). Study of a sand smelt (Atherina boyeri Risso 1810) population reproducing in fresh water. *Ecology of Freshwater Fish*, 1: 77-85.

Rosenfeld J.S. (2002). Functional redundancy in ecology and conservation. *Oikos*, 98: 156-162.

Rotjan R.D. & Lewis S.M. (2008). Impact of coral predators on tropical reefs. *Marine Ecology Progress Series*, 367: 73-91.

Ruitton S., Francour P. & Boudouresque C.F. (2000). Relationships between algae, benthic herbivorous invertebrates and fishes in rocky sublittoral communities of a temperate sea (Mediterranean). *Estuarine, Coastal and Shelf Science*, 50: 217-230.

Sasaki T., Okubo S., Okayasu T., Jamsran U., Ohkuro T. & Takeuchi K. (2009). Two-phase functional redundancy in plant communities along a grazing gradient in Mongolian rangelands. *Ecology*, 90: 2598-2608.

Schindler D.E., Carpenter S.R., Cole J.J., Kitchell J.F. & Pace M.L. (1997). Influence of food web structure on carbon exchange between lakes and the atmosphere. *Science*, 277: 248-251.

Shahi M., Kamrani E., Salehi M., Habibi R. & Hanafi-Bojd A.A. (2015). Native larvivorous fish in an endemic malarious area of southern Iran, a biological alternative factor for chemical larvicides in malaria control program. *Iranian Journal of Public Health*, 44: 1544-1549.

Shears N.T. & Babcock R.C. (2002). Marine reserves demonstrate top-down control of community structure on temperate reefs. *Oecologia*, 132: 131-142.

Shimadzu H., Dornelas M., Henderson P.A. & Magurran A.E. (2013). Diversity is maintained by seasonal variation in species abundance. *BMC Biology*, 11.

Smolders A.J., van der Velde G., Roelofs J.G. & Guerrero Hiza M.A. (2000). El Nino caused collapse of the sabalo fishery (Prochilodus lineatus, Pisces: Prochilodontidae) in a South American river. *Naturwissenschaften*, 87: 30-32.

Strange E.M., Fausch K.D. & Covich A.P. (1999). Sustaining ecosystem services in human-dominated watersheds: biohydrology and ecosystem processes in the South Platte river basin. *Environmental Management*, 24: 39-54.

Taylor B.W., Flecker A.S. & Hall R.O., Jr. (2006). Loss of a harvested fish species disrupts carbon flow in a diverse tropical river. *Science*, 313: 833-836.

TEEB (2010). *The Economics of Ecosystems and Biodiversity: Ecological and Economical Foundations*. Earthscan, London and Washington.

Valiela I., McClelland J., Hauxwell J., Behr P.J., Hersh D. & Foreman K. (1997). Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography*, 42: 1105-1118.

van der Linden P., Marchini A., Dolbeth M., Patrício J., Veríssimo H. & Marques J.C. (2016). The performance of trait-based indices in an estuarine environment. *Ecological Indicators*, 61: 378-389.

Vanni M.J., Bowling A.M., Dickman E.M., Hale R.S., Higgins K.A., Horgan M.J., Knoll L.B., Renwick W.H. & Stein R.A. (2008). Nutrient cycling by fish supports relatively more primary production as lake productivity increases. *Ecology*, 87: 1696-1709.

Vasconcelos R.P., Reis-Santos P., Fonseca V., Maia A., Ruano M., Franca S., Vinagre C., Costa M.J. & Cabral H. (2007). Assessing anthropogenic pressures on estuarine fish nurseries along the Portuguese coast: a multi-metric index and conceptual approach. *Science of the Total Environment*, 374: 199-215.

Vasconcelos R.P., Reis-Santos P., Fonseca V., Ruano M., Tanner S., Costa M.J. & Cabral H.N. (2009). Juvenile fish condition in estuarine nurseries along the Portuguese coast. *Estuarine, Coastal and Shelf Science*, 82: 128-138.

Vasconcelos R.P., Reis-Santos P., Maia A., Fonseca V., França S., Wouters N., Costa M.J. & Cabral H.N. (2010). Nursery use patterns of commercially important marine fish species in estuarine systems along the Portuguese coast. *Estuarine, Coastal and Shelf Science*, 86: 613-624.

Vasconcelos R.P., Reis-Santos P., Tanner S., Maia A., Latkoczy C., Günther D., Costa M.J. & Cabral H. (2008). Evidence of estuarine nursery origin of five coastal fish species along the Portuguese coast through otolith elemental fingerprints. *Estuarine, Coastal and Shelf Science*, 79: 317-327.

Verberk W.C.E.P., van Noordwijk C.G.E. & Hildrew A.G. (2013). Delivering on a promise: integrating species traits to transform descriptive community ecology into a predictive science. *Freshwater Science*, 32: 531-547.

Verdelhos T., Cardoso P.G., Dolbeth M. & Pardal M.A. (2014). Recovery trends of Scrobicularia plana populations after restoration measures, affected by extreme climate events. *Marine Environmental Research*, 98: 39-48.

Verdelhos T., Neto J.M., Marques J.C. & Pardal M.A. (2005). The effect of eutrophication abatement on the bivalve Scrobicularia plana. *Estuarine, Coastal and Shelf Science*, 63: 261-268.

Verheye H.M. & Richardson A.J. (1998). Long-term increase in crustacean zooplankton abundance in the southern Benguela upwelling region (1951–1996): bottom-up or top-down control? *ICES Journal of Marine Science*, 55: 803-807.

Veríssimo H., Lane M., Patrício J., Gamito S. & Marques J.C. (2013). Trends in water quality and subtidal benthic communities in a temperate estuary: Is the response to restoration efforts hidden by climate variability and the Estuarine Quality Paradox? *Ecological Indicators*, 24: 56-67.

Villéger S., Ramos Miranda J., Flores Hernandez D. & Mouillot D. (2012). Low functional beta-diversity despite high taxonomic beta-diversity among tropical estuarine fish communities. *PLoS One*, 7: 1-10.

Vinagre C., Santos F.D., Cabral H.N. & Costa M.J. (2009). Impact of climate and hydrology on juvenile fish recruitment towards estuarine nursery grounds in the context of climate change. *Estuarine, Coastal and Shelf Science*, 85: 479-486.

Violle C., Navas M.L., Vile D., Kazakou E., Fortunel C., Hummel I. & Garnier E. (2007). Let the concept of trait be functional! *Oikos*, 116: 882-892.

Walker B., Kinzig A. & Langridge J. (1999). Plant attribute diversity, resilience, and ecosystem function: the nature and significance of dominant and minor species. *Ecosystems*, 2: 95-113.

Walker B.H. (1992). Biodiversity and ecological redundancy. *Conservation Biology*, 6: 18-23.

Ward J.H. (1963). Hierarchical Grouping to Optimize an Objective Function. *Journal of the American Statistical Association*, 58: 236-244.

Wellnitz T. & Poff N.L. (2001). Functional redundancy in heterogeneous environments: implications for conservation. *Ecology Letters*, 4: 177-179.

Whitfield A.K., Elliott M., Basset A., Blaber S.J.M. & West R.J. (2012). Paradigms in estuarine ecology – A review of the Remane diagram with a suggested revised model for estuaries. *Estuarine, Coastal and Shelf Science*, 97: 78-90.

Whitfield A.K., Taylor R.H., Fox C. & Cyrus D.P. (2006). Fishes and salinities in the St Lucia estuarine system—a review. *Reviews in Fish Biology and Fisheries*, 16: 1-20.

Woodward G., Ebenman B., Emmerson M., Montoya J.M., Olesen J.M., Valido A. & Warren P.H. (2005). Body size in ecological networks. *Trends in Ecology and Evolution*, 20: 402-409.

Worm B., Barbier E.B., Beaumont N., Duffy J.E., Folke C., Halpern B.S., Jackson J.B., Lotze H.K., Micheli F., Palumbi S.R., Sala E., Selkoe K.A., Stachowicz J.J. & Watson R. (2006). Impacts of biodiversity loss on ocean ecosystem services. *Science*, 314: 787-790.

Xie P. & Liu J. (2001). Practical success of biomanipulation using filter-feeding fish to control cyanobacteria blooms: a synthesis of decades of research and application in a subtropical hypereutrophic lake. *The Scientific World Journal*, 1: 337-356.

Yachi S. & Loreau M. (1999). Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*, 96: 1463-1468.

Zhou H.Y., Cheung R.Y.H. & Wong M.H. (1999). Bioaccumulation of organochlorines in freshwater fish with different feeding modes cultured in treated wastewater. *Water Research*, 33: 2747-2756.

CHAPTER 3

Final Remarks

Final Remarks

The results of this study reveal new perspectives on the value of biodiversity to human society, and also represent an important step towards the quantification of the contribution of fish communities to ecosystem services, particularly in estuaries, although this goal needs further research. The concept of ecosystem services is fundamental in this context, since loss of species can cause the disappearance of essential functions and therefore affect the provision of ecosystem services to human populations (Elmqvist *et al.* 2003; Díaz *et al.* 2006).

This study showed that an estuarine fish assemblage can potentially mediate the provision of regulating ecosystem services, such as nutrient cycling (which allows the presence of nutrients in the water), biological control (important for a wide range of aspects, from regulation of food web dynamics to pest regulation and control of invasive species), maintenance of sediment processes and sediment redistribution (which influence the structure of the substrate and sediment quality) and regulation of linkages between ecosystems (relevant to transfer energy, nutrients and genetic material to other regions, especially considering that estuaries are transitional systems). Also, by estimating functional redundancy of fish functional groups by season, station and year, this study investigated possible drivers of its variation and highlighted the need to assess the resilience of ecosystem services, which contributes to create a better understanding of the relevance of conservation of fish assemblages. Moreover, the maintenance of functional characteristics within fish assemblages in estuaries is critical to ensure the healthy functioning of these transitional systems, especially considering the range of pressures which affect them, both from natural and anthropogenic sources (Borja *et al.* 2010). Further research on fish communities should be developed, directly linking their functional traits to contributions to the provision of ecosystem services, since this group of organisms is poorly studied in this context and also extremely impacted by fisheries and habitat and environmental alterations. This approach characterized the connection between fish functional traits and ecosystem functions based on publicly available information referring to all ecosystems and aquatic habitats, throughout the world. It is necessary to investigate those connections in estuaries, since available information in these systems is still scarce, concerning links between fish functional traits and ecosystem functioning (Pinto *et al.* 2010).

Future studies should develop the quantification of services provided by fish, mainly considering two perspectives: ecological (Holmlund & Hammer 1999; Pratchett *et al.* 2014) and economical (Pinto *et al.* 2010; de Jonge *et al.* 2012). Furthermore, to measure the contribution of fish to ecosystem services in estuarine context, other direct and indirect ways should be explored, besides measuring functional traits. For example, to assess the role of fish in nutrient cycling, Allgeier *et al.* (2013) estimated the excretion rate of phosphorus and nitrogen by fish, which increases the availability of nutrients in aquatic environments and supports primary production (Holmlund & Hammer 1999; McIntyre *et al.* 2007). Additionally, Cardinale *et al.* (2012) suggested that climate regulation by organisms could be quantified by: 1 - the net influence of biodiversity on photosynthesis (exchange of CO_2 for O_2); 2 – the accumulation of carbon in live plant tissue; 3 – herbivory (plant carbon ingested by herbivorous animals); 4 – decomposition (carbon resulting from death and decomposition of organisms, returning to the atmosphere). However, this raises several problems, especially concerning the specific role of fish in the provision of this ecosystem service, since the relationship between fish traits and for example climate regulation is indirect and the attempts in quantifying it are still poorly developed (Schindler *et al.* 1997;

Holmlund & Hammer 1999). Thus, further research is needed to develop methods of quantification of the contribution of fish to ecosystem functioning and services, especially in estuaries.

The effects of anthropogenic pressures in fish communities are widely studied (Kennish 2002; Yuksek *et al.* 2006; Vasconcelos *et al.* 2007; Henriques *et al.* 2013), which is important to recognize the factors affecting their variation and also to determine what should be done to mitigate negative impacts. However, it is critical to investigate the effects of anthropogenic pressures in the functional composition of fish communities and thus in ecosystem functioning (Kennish 2002). For example, Jennings and Lock (1996) explored the effects of reef fishing in fish communities, reporting changes in size, behavior and community structure, which have direct influence in functional composition and thus in the performance of important functions, such as grazing on reef algae by herbivorous fishes, which avoids algae outgrowth and enables coral settlement (Potts 1977; Brock 1979). Also, Pinnegar *et al.* (2000) highlighted that one of the indirect effects of fishing is the occurrence of trophic cascades, describing some examples in the Mediterranean, such as the depletion of invertebrate-feeding fish that control the populations of sea urchins (Sala & Zabala 1996), which remove large erect algae (an important structural habitat for invertebrates), and induce the formation of coralline barrens (Lawrence 1975). For example, in the Gulf of Maine, large predatory finfish are absent due to overfishing of cod (*Gadus morhua*), which altered food webs in this region (Steneck 1997). Therefore, it is essential to develop research about anthropogenic effects in ecosystem functioning and services, especially in estuaries and concerning fish.

When assessing the provision of ecosystem services, there are several possible approaches and each presents some disadvantages. The approach of functional groups used in this study was informative (Dumay *et al.* 2004) but it has limitations, for instance the definition of groups tends to be site/context specific and it needs empirical exploration, especially concerning the mechanisms by which species affect ecosystem functioning (Murray *et al.* 2014). Also, the assignment of species into functional groups assumes they are totally equivalent (Carmona *et al.* 2016), which is not necessarily true, since species can share traits but interact differently with the environment, as observed by Resetarits and Chalcraft (2007) for three congeneric fish species. Thus, the interactions between and within species, such as competition and mutualism, must be taken into account for service provision, especially considering functional groups (Luck *et al.* 2009).

In estuaries, different types of habitats are generally present and interconnected (França *et al.* 2009). In this study, the Mondego estuary was assessed as a whole, without differentiating between habitats, only between a salinity gradient. However, that would be an important aspect for research, since it is known that fish assemblages show variation along different habitat types within estuaries (Pihl *et al.* 2002; França *et al.* 2009), according to food availability, environmental conditions and predator abundance, among other factors (Blaber & Blaber 1980). For example, salt marsh habitat supports different fish assemblages than seagrass beds (França *et al.* 2009) which have lower predator pressure and serve as a nursery for juvenile marine fish species, such as *Diplodus vulgaris* (Gray *et al.* 1998; França *et al.* 2009). Therefore, it is important to understand the differences in fish communities, especially in a functional level, among the range of habitats in estuaries, in order to understand potential differences in ecosystem functioning and services and predict the effects of habitat degradation (Connolly *et al.* 2005).

Given that estuaries support fish species from different aquatic ecosystems, assessing the contribution of fish to ecosystem services in estuaries can help understanding their contribution in other aquatic systems, transposing the knowledge about the mechanistic links between functional structure and the provision of services. Additionally, the present study improves understanding of how fish mediate regulating functions in marine and freshwater ecosystems. However, further research on this issue is still

needed as fish assemblages diverge according to environmental and biotic factors (e.g. structure of food webs, number of species within each functional trait, habitat structure) (Elliott & Dewailly 1995; Harmelin-Vivien *et al.* 2001; Jackson *et al.* 2001a; Magalhães *et al.* 2002; Martino & Able 2003). Yet, they can share functional roles and therefore contribute to the same ecosystem services, thus making research about this subject more interesting.

Considering that estuaries constitute transitional systems that connect marine and freshwater environments (Beck *et al.* 2001; Vasconcelos *et al.* 2015), the healthy functioning of estuarine systems is essential to maintain the linkages between rivers and oceans, and also support the diversity of fish species within and between these systems. In this context, it will be important to assess the provision of ecosystem services in these different aquatic systems and their relationships and interactions (Bennett *et al.* 2009). Several studies have already been conducted to assess the delivery of ecosystem services by single types of aquatic systems, such as freshwater (Jackson *et al.* 2001b), marine (Beaumont *et al.* 2007; Liquete *et al.* 2013; Hattam *et al.* 2015) and estuarine (Barbier *et al.* 2011) systems, but it would be interesting to investigate the relationship between these systems and the services they provide. Also, the creation of a common framework of ecosystem services by aquatic systems, representing the interactions between them, would be useful for management and conservation purposes (Costanza *et al.* 1997).

Although this study focuses on the links between fish functional groups and the delivery of ecosystem services, there are other groups of organisms involved in mediating this delivery, by performing essential functions and interacting with fish in aquatic environments. For example, bioturbation is an ecosystem function that is involved in the maintenance of sediment processes, an important ecosystem service provided in the Mondego estuary and other aquatic systems (Holmlund & Hammer 1999), which is accomplished by fish but especially by macroinvertebrates (Gerino *et al.* 2003; Adámek & Maršálek 2013). Also, primary producers contribute directly and strongly to carbon fixation, which is essential to climate regulation (de Bello *et al.* 2010; Lavorel & Grigulis 2012), an ecosystem service also provided by fish in an indirect way, by suppressing zooplankton in lakes and therefore releasing phytoplankton from grazing pressure (Schindler *et al.* 1997). Nutrient cycling is another example of ecosystem services being mediated by several groups of organisms, such as algae, fish and aquatic insects (Flecker *et al.* 2002; Vanni *et al.* 2008; Macadam & Stockan 2015). As such, interactions between fish and other groups of organisms are extremely important to be considered in ecosystem services provision (Balvanera *et al.* 2005) and thus should be further investigated.

The effects of environmental variables in variation of functional traits were explored in this study, as well as temporal, spatial and seasonal variation of the fish functional groups, but in order to fully understand the drivers of change in this fish assemblage, it would be also necessary to address the anthropogenic pressures in the Mondego estuary, such as industrial pollution, eutrophication and overfishing, and how they affect the environmental conditions in the estuary (Martinho *et al.* 2008; Dolbeth *et al.* 2013). It is already known that anthropogenic impacts on the hydro-morphology of estuaries can cause changes in fish communities (Baptista *et al.* 2015), making it crucial to integrate anthropogenic stressors with natural stressors in these assessments (Pinto *et al.* 2014).

Overall, this study highlighted the contribution of fish to the delivery of regulating ecosystem services and estimated the resilience of those services through functional redundancy, which is an important step towards conservation. It is necessary to consider ecosystem services in decision-making (Daily *et al.* 2009), especially in estuaries. Also, by assessing functional redundancy, we gained knowledge of the sensitivity of fish functional groups to disturbance (Muntadas *et al.* 2016), which is an important tool in the correct management of fish resources in this estuary. Finally, the results of this study can be transposed

to other estuarine systems, although further investigation is needed, especially concerning the ecosystem services that fish assemblages can provide.

Literature cited

Adámek Z. & Maršálek B. (2013). Bioturbation of sediments by benthic macroinvertebrates and fish and its implication for pond ecosystems: a review. *Aquaculture International*, 21: 1-17.

Allgeier J.E., Yeager L.A. & Layman C.A. (2013). Consumers regulate nutrient limitation regimes and primary production in seagrass ecosystems. *Ecology*, 94: 521-529.

Balvanera P., Kremen C. & Martínez-Ramos M. (2005). Applying community structure analysis to ecosystem function: examples from pollination and carbon storage. *Ecological Applications*, 15: 360-375.

Baptista J., Martinho F., Nyitrai D., Pardal M.A. & Dolbeth M. (2015). Long-term functional changes in an estuarine fish assemblage. *Marine Pollution Bulletin*, 97: 125-134.

Barbier E.B., Hacker S.D., Kennedy C., Koch E.W., Stier A.C. & Silliman B.R. (2011). The value of estuarine and coastal ecosystem services. *Ecological Monographs*, 81: 169-193.

Beaumont N.J., Austen M.C., Atkins J.P., Burdon D., Degraer S., Dentinho T.P., Derous S., Holm P., Horton T., van Ierland E., Marboe A.H., Starkey D.J., Townsend M. & Zarzycki T. (2007). Identification, definition and quantification of goods and services provided by marine biodiversity: implications for the ecosystem approach. *Marine Pollution Bulletin*, 54: 253-265.

Beck M.W., Heck K.L., Able K.W., Childers D.L., Eggleston D.B., Gillanders B., Halpern B., Hays C.G., Hoshino K., Minello T.J., Orth R.J., Sheridan P.F. & Weinstein M. (2001). The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *BioScience*, 51: 633-641.

Bennett E.M., Peterson G.D. & Gordon L.J. (2009). Understanding relationships among multiple ecosystem services. *Ecology Letters*, 12: 1394-1404.

Blaber S.J.M. & Blaber T.G. (1980). Factors affecting the distribution of juvenile estuarine and inshore fish. *Journal of Fish Biology*, 17: 143-162.

Borja A., Dauer D.M., Elliott M. & Simenstad C.A. (2010). Medium- and long-term recovery of estuarine and coastal ecosystems: patterns, rates and restoration effectiveness. *Estuaries and Coasts*, 33: 1249-1260.

Brock R.E. (1979). An experimental study on the effects of grazing by parrotfishes and role of refuges in benthic community structure. *Marine Biology*, 51: 381-388.

Cardinale B.J., Duffy J.E., Gonzalez A., Hooper D.U., Perrings C., Venail P., Narwani A., Mace G.M., Tilman D., Wardle D.A., Kinzig A.P., Daily G.C., Loreau M., Grace J.B., Larigauderie A., Srivastava D.S. & Naeem S. (2012). Biodiversity loss and its impact on humanity. *Nature*, 486: 59-67.

Carmona C.P., de Bello F., Mason N.W. & Leps J. (2016). Traits Without Borders: Integrating Functional Diversity Across Scales. *Trends in Ecology and Evolution*, 31: 382-394.

Connolly S.R., Hughes T.P., Bellwood D.R. & Karlson R.H. (2005). Community structure of corals and reef fishes at multiple scales. *Science*, 309: 1363-1365.

Costanza R., dArge R., deGroot R., Farber S., Grasso M., Hannon B., Limburg K., Naeem S., ONeill R.V., Paruelo J., Raskin R.G., Sutton P. & vandenBelt M. (1997). The value of the world's ecosystem services and natural capital. *Nature*, 387: 253-260.

Daily G.C., Polasky S., Goldstein J., Kareiva P.M., Mooney H.A., Pejchar L., Ricketts T.H., Salzman J. & Shallenberger R. (2009). Ecosystem services in decision making: time to deliver. *Frontiers in Ecology and the Environment*, 7: 21-28.

de Bello F., Lavorel S., Díaz S., Harrington R., Cornelissen J.H.C., Bardgett R.D., Berg M.P., Cipriotti P., Feld C.K., Hering D., Martins da Silva P., Potts S.G., Sandin L., Sousa J.P., Storkey J., Wardle D.A. & Harrison P.A. (2010). Towards an assessment of multiple ecosystem processes and services via functional traits. *Biodiversity and Conservation*, 19: 2873-2893.

de Jonge V.N., Pinto R. & Turner R.K. (2012). Integrating ecological, economic and social aspects to generate useful management information under the EU Directives' 'ecosystem approach'. *Ocean & Coastal Management*, 68: 169-188.

Díaz S., Fargione J., Chapin F.S., 3rd & Tilman D. (2006). Biodiversity loss threatens human well-being. *PLoS Biology*, 4: 1300-1305.

Dolbeth M., Cardoso P., Grilo T., Raffaelli D. & Pardal M.A. (2013). Drivers of estuarine benthic species distribution patterns following a restoration of a seagrass bed: a functional trait analyses. *Marine Pollution Bulletin*, 72: 47-54.

Dumay O., Tari P.S., Tomasini A. & Mouillot D. (2004). Functional groups of lagoon fish species in Languedoc Roussillon, southern France. *Journal of Fish Biology*, 64: 970-983.

Elliott M. & Dewailly F. (1995). The structure and components of european estuarine fish assemblages. *Netherlands Journal of Aquatic Ecology*, 29: 397 - 417.

Elmqvist T., Folke C., Nyström M., Peterson G., Bengtsson J., Walker B. & Norberg J. (2003). Response diversity, ecosystem change, and resilience. *Frontiers in Ecology and the Environment*, 1: 488-494.

Flecker A.S., Taylor B.W., Bernhardt E.S., Hood J.M., Cornwell W.K., Cassatt S.R., Vanni M.J. & Altman N.S. (2002). Interactions between herbivorous fishes and limiting nutrients in a tropical stream ecosystem. *Ecology*, 83: 1831-1844.

França S., Costa M.J. & Cabral H.N. (2009). Assessing habitat specific fish assemblages in estuaries along the Portuguese coast. *Estuarine, Coastal and Shelf Science*, 83: 1-12.

Gerino M., Stora G., François-Carcaillet F., Gilbert F., Poggiale J.-C., Mermillod-Blondin F., Desrosiers G. & Vervier P. (2003). Macro-invertebrates functional groups in freshwater and marine sediments: a commun mechanistic classification. *Vie et Milieu*: 221-231.

Gray C.A., Chick R.C. & McElligott D.J. (1998). Diel changes in assemblages of fishes associated with shallow seagrass and bare sand. *Estuarine, Coastal and Shelf Science*, 46: 849-859.

Harmelin-Vivien M.L., Harmelin J.-G. & Almeida A.J. (2001). Structure of fish assemblages on coastal rocky shores of the Azores. *Boletim do Museu Municipal do Funchal*, 6: 127-138.

Hattam C., Atkins J.P., Beaumont N., Borger T., Bohnke-Henrichs A., Burdon D., de Groot R., Hoefnagel E., Nunes P.A.L.D., Piwowarczyk J., Sastre S. & Austen M.C. (2015). Marine ecosystem services: Linking indicators to their classification. *Ecological Indicators*, 49: 61-75.

Henriques S., Pais M.P., Batista M.I., Costa M.J. & Cabral H.N. (2013). Response of fish-based metrics to anthropogenic pressures in temperate rocky reefs. *Ecological Indicators*, 25: 65-76.

Holmlund C.M. & Hammer M. (1999). Ecosystem services generated by fish populations. *Ecological Economics*, 29: 253-268.

Jackson D.A., Peres-Neto P.R. & Olden J.D. (2001a). What controls who is where in freshwater fish communities - the roles of biotic, abiotic and spatial factors. *Canadian Journal of Fisheries and Aquatic Sciences*, 58: 157-170.

Jackson R.B., Carpenter S.R., Dahm C.N., McKnight D.M., Naiman R.J., Postel S.L. & Running S.W. (2001b). Water in a changing world. *Ecological Applications*, 11: 1027-1045.

Jennings S. & Lock J.M. (1996). Population and ecosystem effects of reef fishing. In: *Reef Fisheries* (eds. Polunin NVC & Roberts CM). Chapman and Hall London, U. K. , pp. 193 - 218.

Kennish M.J. (2002). Environmental threats and environmental future of estuaries. *Environmental Conservation*, 29: 78-107.

Lavorel S. & Grigulis K. (2012). How fundamental plant functional trait relationships scale-up to trade-offs and synergies in ecosystem services. *Journal of Ecology*, 10.1111/j.1365-2745.2011.01914.x.

Lawrence J.M. (1975). On the relationships between marine plants and sea urchins. *Oceanography and Marine Biology: an Annual Review*, 13: 213-286.

Liquete C., Piroddi C., Drakou E.G., Gurney L., Katsanevakis S., Charef A. & Egoh B. (2013). Current status and future prospects for the assessment of marine and coastal ecosystem services: a systematic review. *PLoS One*, 8: 1-15.

Luck G.W., Harrington R., Harrison P.A., Kremen C., Berry P.M., Bugter R., Dawson T.P., de Bello F., Diaz S., Feld C.K., Haslett J.R., Hering D., Kontogianni A., Lavorel S., Rounsevell M., Samways M.J., Sandin L., Settele J., Sykes M.T., van den Hove S., Vandewalle M. & Zobel M. (2009). Quantifying the Contribution of Organisms to the Provision of Ecosystem Services. *BioScience*, 59: 223-235.

Macadam C.R. & Stockan J.A. (2015). More than just fish food: ecosystem services provided by freshwater insects. *Ecological Entomology*, 40: 113-123.

Magalhães M.F., Batalha D.C. & Collares-Pereira M.J. (2002). Gradients in stream fish assemblages across a Mediterranean landscape: contributions of environmental factors and spatial structure. *Freshwater Biology*, 47: 1015-1031.

Martinho F., Viegas I., Dolbeth M., Leitão R., Cabral H.N. & Pardal M.A. (2008). Assessing estuarine environmental quality using fish-based indices: performance evaluation under climatic instability. *Marine Pollution Bulletin*, 56: 1834-1843.

Martino E.J. & Able K.W. (2003). Fish assemblages across the marine to low salinity transition zone of a temperate estuary. *Estuarine, Coastal and Shelf Science*, 56: 969-987.

McIntyre P.B., Jones L.E., Flecker A.S. & Vanni M.J. (2007). Fish extinctions alter nutrient recycling in tropical freshwaters. *Proceedings of the National Academy of Sciences of the United States of America*, 104: 4461-4466.

Muntadas A., de Juan S. & Demestre M. (2016). Assessing functional redundancy in chronically trawled benthic communities. *Ecological Indicators*, 61: 882-892.

Murray F., Douglas A. & Solan M. (2014). Species that share traits do not necessarily form distinct and universally applicable functional effect groups. *Marine Ecology Progress Series*, 516: 23-34.

Pihl L., Cattrijsse A., Codling I., Mathieson S., McLusky D.S. & Roberts C. (2002). Habitat use by fishes in estuaries and other brackish areas. In: *Fishes in Estuaries* (eds. Elliott M & Hemingway K). Blackwell Publishing Ltd.

Pinnegar J.K., Polunin N.V.C., Francour P., Badalamenti F., Chemello R., Harmelin-Vivien M., Hereu B., Milazzo M., Zabala M., D'Anna G. & Pipitone C. (2000). Trophic cascades in benthic marine ecosystems: lessons for fisheries and protected-area management. *Environmental Conservation*, 27: 179 - 200.

Pinto R., de Jonge V.N. & Marques J.C. (2014). Linking biodiversity indicators, ecosystem functioning, provision of services and human well-being in estuarine systems: application of a conceptual framework. *Ecological Indicators*, 36: 644-655.

Pinto R., Patrício J., Neto J.M., Salas F. & Marques J.C. (2010). Assessing estuarine quality under the ecosystem services scope: Ecological and socioeconomic aspects. *Ecological Complexity*, 7: 389-402.

Potts D.C. (1977). Suppression of coral populations by filamentous algae within damselfish territories. *Journal of Experimental Marine Biology and Ecology*, 28: 207-216.

Pratchett M.S., Hoey A.S. & Wilson S.K. (2014). Reef degradation and the loss of critical ecosystem goods and services provided by coral reef fishes. *Current Opinion in Environmental Sustainability*, 7: 37-43.

Resetarits W.J. & Chalcraft D.R. (2007). Functional diversity within a morphologically conservative genus of predators: implications for functional equivalence and redundancy in ecological communities. *Functional Ecology*, 21: 793-804.

Sala E. & Zabala M. (1996). Fish predation and the structure of the sea urchin Paracentrotus lividus populations in the NW Mediterranean. *Marine Ecology Progress Series*, 140: 71-81.

Schindler D.E., Carpenter S.R., Cole J.J., Kitchell J.F. & Pace M.L. (1997). Influence of food web structure on carbon exchange between lakes and the atmosphere. *Science*, 277: 248-251.

Steneck R.S. (1997). Fisheries-induced biological changes to the structure and function of the Gulf of Maine ecosystem. Plenary paper. In: *Proceedings of the Gulf of Maine Ecosystem Dynamics Scientific Symposium and Workshop* (eds. Wallace GT & Braasch EF). Regional Association for Research on the Gulf of Maine Hanover, NH, pp. 151-165.

Vanni M.J., Bowling A.M., Dickman E.M., Hale R.S., Higgins K.A., Horgan M.J., Knoll L.B., Renwick W.H. & Stein R.A. (2008). Nutrient cycling by fish supports relatively more primary production as lake productivity increases. *Ecology*, 87: 1696-1709.

Vasconcelos R.P., Henriques S., França S., Pasquaud S., Cardoso I., Laborde M. & Cabral H.N. (2015). Global patterns and predictors of fish species richness in estuaries. *Journal of Animal Ecology*, 84: 1331-1341.

Vasconcelos R.P., Reis-Santos P., Fonseca V., Maia A., Ruano M., Franca S., Vinagre C., Costa M.J. & Cabral H. (2007). Assessing anthropogenic pressures on estuarine fish nurseries along the Portuguese coast: a multi-metric index and conceptual approach. *Science of the Total Environment*, 374: 199-215.

Yuksek A., Okus E., Yilmaz I.N., Aslan-Yilmaz A. & Tas S. (2006). Changes in biodiversity of the extremely polluted Golden Horn Estuary following the improvements in water quality. *Marine Pollution Bulletin*, 52: 1209-1218.

Appendix I

Table 1 – Characterization of the seven fish functional groups generated by hierarchical cluster analysis of the Mondego estuary fish assemblage. Shown is the percentage of each trait category in each functional group (measured in biomass): the total of one hundred percent is represented by the whole assemblage, i.e. the sum of percentage of all categories in a given trait across the seven groups.

	Ecosystem services provider groups (percentage of biomass of species)						
Variables	Group 1 (2.23%)	Group 2 (6.33%)	Group 3 (3.63%)	Group 4 (0.72%)	Group 5 (32.06%)	Group 6 (53.33%)	Group 7 (1.70%)
Prominent families	Syngnathidae	Sparidae, Congridae, Scophthalmidae	Soleidae	Gadidae, Atherinidae	Gobiidae, Soleidae	Mugilidae	Poeciliidae, Cyprinidae
Mobility							
High	0.00%	6.33%	0.88%	0.70%	31.54%	52.89%	0.00%
Medium	2.00%	0.00%	2.75%	0.00%	0.01%	0.44%	0.02%
Sedentary	0.23%	0.00%	0.00%	0.01%	0.00%	0.00%	1.68%
Territorial	0.00%	0.00%	0.00%	0.00%	0.51%	0.00%	0.00%
Body size							
Large	0.00%	5.59%	0.00%	0.00%	0.00%	31.12%	1.68%
Medium	2.22%	0.00%	2.16%	0.00%	30.35%	21.74%	0.00%
Small	0.00%	0.74%	1.47%	0.72%	0.73%	0.00%	0.02%
Very small	0.01%	0.00%	0.00%	0.00%	0.99%	0.47%	0.00%
Diet							
Planktivorous	0.00%	0.00%	0.00%	0.00%	0.31%	0.00%	0.00%
Invertebrate feeder	0.00%	0.00%	3.63%	0.22%	31.76%	49.70%	0.00%
Omnivorous	0.62%	4.65%	0.00%	0.50%	0.00%	0.74%	1.70%
Macrocarnivorous	1.61%	1.67%	0.00%	0.00%	0.00%	0.00%	0.00%
Detritivorous	0.00%	0.00%	0.00%	0.00%	0.00%	2.89%	0.00%
Feeding mode							
Browser	0.00%	4.65%	3.12%	0.00%	31.99%	53.33%	1.70%
Filterer	0.00%	0.00%	0.00%	0.00%	0.07%	0.00%	0.00%
Hunter	2.23%	1.67%	0.51%	0.72%	0.00%	0.00%	0.00%
Salinity preference							
Marine	0.93%	2.63%	1.51%	0.30%	13.33%	22.18%	0.00%
Brackish	0.35%	0.00%	0.00%	0.30%	13.33%	22.18%	0.00%
Freshwater	0.00%	0.00%	0.00%	0.06%	0.02%	22.18%	0.71%

Table 2 – Characterization of the seven fish functional groups generated by hierarchical cluster analysis of the Mondego estuary fish assemblage. Shown is the percentage of each trait category in each functional group (measured in density): the total of one hundred percent is represented by the whole assemblage, i.e. the sum of percentage of all categories in a given trait across the seven groups.

	Ecosystem services provider groups (percentage of density of species)						
Variables	Group 1 (1.33%)	Group 2 (4.07%)	Group 3 (1.36%)	Group 4 (3.01%)	Group 5 (24.82%)	Group 6 (65.29%)	Group 7 (0.12%)
Prominent families	Syngnathidae	Sparidae, Congridae, Scophthalmidae	Soleidae	Gadidae, Atherinidae	Gobiidae, Soleidae	Mugilidae	Poeciliidae, Cyprinidae
Mobility							
High	0.00%	4.07%	0.39%	2.34%	23.61%	65.20%	0.00%
Medium	0.63%	0.00%	0.96%	0.00%	0.04%	0.08%	0.10%
Sedentary	0.70%	0.00%	0.00%	0.67%	0.01%	0.00%	0.02%
Territorial	0.00%	0.00%	0.00%	0.00%	1.16%	0.00%	0.00%
Body size							
Large	0.00%	3.77%	0.00%	0.00%	0.00%	28.67%	0.02%
Medium	1.30%	0.00%	0.55%	0.00%	10.43%	10.60%	0.00%
Small	0.00%	0.29%	0.81%	3.01%	1.74%	0.00%	0.07%
Very small	0.04%	0.00%	0.01%	0.00%	12.65%	26.02%	0.03%
Diet							
Planktivorous	0.00%	0.00%	0.00%	0.00%	0.97%	0.00%	0.00%
Invertebrate feeder	0.01%	0.00%	1.36%	2.86%	23.85%	63.75%	0.00%
Omnivorous	0.05%	3.92%	0.00%	0.16%	0.00%	0.11%	0.12%
Macrocarnivorous	1.28%	0.15%	0.00%	0.00%	0.00%	0.00%	0.00%
Detritivorous	0.00%	0.00%	0.00%	0.00%	0.00%	1.43%	0.00%
Feeding mode							
Browser	0.00%	3.92%	1.16%	0.00%	24.65%	65.29%	0.09%
Filterer	0.00%	0.00%	0.00%	0.00%	0.16%	0.00%	0.00%
Hunter	1.33%	0.15%	0.20%	3.01%	0.01%	0.00%	0.03%
Salinity preference							
Marine	0.51%	1.55%	0.52%	1.15%	9.47%	24.92%	0.00%
Brackish	0.27%	0.00%	0.00%	1.15%	9.47%	24.92%	0.01%
Freshwater	0.00%	0.00%	0.00%	0.97%	0.13%	24.92%	0.04%

Table 3 – Characterization of the seven fish functional groups generated by hierarchical cluster analysis of the Mondego estuary fish assemblage. Shown is the percentage of each trait category in each functional group (measured in number of species): the total of one hundred percent is represented by the whole assemblage, i.e. the sum of percentage of all categories in a given trait across the seven groups.

	Ecosystem services provider groups (percentage of species)						
Variables	Group 1 (11.63%)	Group 2 (9.30%)	Group 3 (20.93%)	Group 4 (11.63%)	Group 5 (20.93%)	Group 6 (18.60%)	Group 7 (6.98%)
Prominent families	Syngnathidae	Sparidae, Congridae, Scophthalmidae	Soleidae	Gadidae, Atherinidae	Gobiidae, Soleidae	Mugilidae	Poeciliidae, Cyprinidae
Mobility							
High	0.00%	9.30%	4.65%	9.30%	9.30%	16.28%	0.00%
Medium	4.65%	0.00%	13.95%	0.00%	2.33%	2.33%	4.65%
Sedentary	6.98%	0.00%	0.00%	2.33%	2.33%	0.00%	2.33%
Territorial	0.00%	0.00%	2.33%	0.00%	6.98%	0.00%	0.00%
Body size							
Large	0.00%	4.65%	0.00%	0.00%	0.00%	4.65%	2.33%
Medium	6.98%	0.00%	4.65%	0.00%	2.33%	11.63%	0.00%
Small	0.00%	4.65%	11.63%	9.30%	13.95%	0.00%	2.33%
Very small	4.65%	0.00%	4.65%	2.33%	4.65%	2.33%	2.33%
Diet							
Planktivorous	0.00%	0.00%	0.00%	0.00%	11.63%	0.00%	0.00%
Invertebrate feeder	2.33%	0.00%	20.93%	6.98%	9.30%	9.30%	0.00%
Omnivorous	2.33%	4.65%	0.00%	4.65%	0.00%	4.65%	6.98%
Macrocarnivorous	6.98%	4.65%	0.00%	0.00%	0.00%	0.00%	0.00%
Detritivorous	0.00%	0.00%	0.00%	0.00%	0.00%	4.65%	0.00%
Feeding mode							
Browser	0.00%	4.65%	16.28%	0.00%	16.28%	18.60%	4.65%
Filterer	0.00%	0.00%	0.00%	0.00%	2.33%	0.00%	0.00%
Hunter	11.63%	4.65%	4.65%	11.63%	2.33%	0.00%	2.33%
Salinity preference							
Marine	6.33%	5.06%	11.39%	6.33%	11.39%	10.13%	0.00%
Brackish	2.53%	0.00%	0.00%	6.33%	11.39%	10.13%	1.27%
Freshwater	0.00%	0.00%	0.00%	2.53%	1.27%	10.13%	3.80%

Appendix II

Table 1 – PERMANOVA results from the pair-wise tests for the differences in biomass of each functional group considering factors season, station and year. Each value corresponds to the t statistic and the significance is in superscript (* p-value < 0.05, ns - non-significant). Blank cells correspond to non-significant main tests.

Factor	Levels	Total Biomass	Biomass G1	Biomass G2	Biomass G3	Biomass G4	Biomass G5	Biomass G6	Biomass G7
Season	summer, autumn	1.173^{ns}	-	3.58*	3.357*	2.794*	-	-	-
	summer, winter	1.925*	-	3.052*	3.107*	2.505*	-	-	-
	summer, spring	0.752^{ns}	-	1.102^{ns}	1.225^{ns}	1.106^{ns}	-	-	-
	autumn, winter	2.975*	-	1.554^{ns}	0.873^{ns}	0.539^{ns}	-	-	-
	autumn, spring	0.99^{ns}	-	3.002*	1.795*	3.111*	-	-	-
	winter, spring	2.33*	-	2.397*	1.627^{ns}	2.937*	-	-	-
Station	M, S1	8.296*	1.498*	0.716*	2.673*	0.593*	7.211*	5.192*	-
	M, S2	5.813*	2.150*	0.786*	1.108*	0.289*	6.012*	2.071*	-
	M, N1	2.269*	5.071^{ns}	1.020*	1.597*	3.736*	2.410*	1.208^{ns}	-
	M, N2	1.873*	1.725*	2.650*	0.527^{ns}	0.667*	3.087*	2.642*	-
	S1, S2	6.037*	0.729*	0.581^{ns}	2.509^{ns}	0.391^{ns}	3.035*	6.701*	-
	S1, N1	8.536*	2.779^{ns}	1.506^{ns}	3.013^{ns}	2.611^{ns}	6.688*	6.514*	-
	S1, N2	7.278*	1.183^{ns}	2.678^{ns}	1.018^{ns}	0.438^{ns}	4.771*	8.139*	-
	S2, N1	4.709*	2.223*	1.519^{ns}	0.991^{ns}	2.199^{ns}	4.671*	1.438^{ns}	-
	S2, N2	3.877*	1.134^{ns}	2.382^{ns}	0.746^{ns}	0.306^{ns}	2.773*	4.029*	-
	N1, N2	0.019^{ns}	1.139^{ns}	2.618^{ns}	1.006^{ns}	3.261^{ns}	1.393^{ns}	2.425*	-
Year	2003, 2004	1.016^{ns}	-	0.731^{ns}	-	0.939^{ns}	1.477^{ns}	1.060^{ns}	-
	2003, 2005	1759^{ns}	-	0.599^{ns}	-	2.003*	0.668^{ns}	2.429^{ns}	-
	2003, 2006	2.215*	-	0.792^{ns}	-	0.979^{ns}	0.842^{ns}	1.636*	-
	2003, 2007	1.627^{ns}	-	0.683^{ns}	-	1.071^{ns}	0.479^{ns}	2.924*	-
	2003, 2008	0.699^{ns}	-	0.914^{ns}	-	1.237^{ns}	0.939^{ns}	1.063^{ns}	-
	2003, 2009	0.798^{ns}	-	0.523^{ns}	-	0.509^{ns}	1.217^{ns}	1.623^{ns}	-
	2003, 2010	1.408^{ns}	-	1.785^{ns}	-	1.359^{ns}	0.574^{ns}	1.980^{ns}	-
	2003, 2011	2.336*	-	1.124^{ns}	-	1.462^{ns}	0.764^{ns}	0.872*	-
	2003, 2012	2.577*	-	0.767^{ns}	-	0.635^{ns}	1.452^{ns}	0.964*	-
	2003, 2013	1.794*	-	2.349*	-	0.515^{ns}	3.078*	1.226^{ns}	-
	2004, 2005	1.457^{ns}	-	0.370^{ns}	-	1.300^{ns}	0.962^{ns}	1.793^{ns}	-
	2004, 2006	1.358^{ns}	-	0.683^{ns}	-	1.498^{ns}	0.834^{ns}	1.977^{ns}	-
	2004, 2007	1.122^{ns}	-	0.608^{ns}	-	0.522^{ns}	1.386^{ns}	2.542*	-
	2004, 2008	0.816^{ns}	-	1.089^{ns}	-	0.762^{ns}	0.870^{ns}	0.528^{ns}	-
	2004, 2009	1.449^{ns}	-	0.709^{ns}	-	0.337^{ns}	1.596^{ns}	0.959^{ns}	-
	2004, 2010	0.875^{ns}	-	2.012*	-	1.586^{ns}	1.148^{ns}	1.542^{ns}	-
	2004, 2011	1.771*	-	1.261^{ns}	-	2.045^{ns}	1.010^{ns}	0.518*	-

Table 1 (continued).

Factor	Levels	Total Biomass	Biomass G1	Biomass G2	Biomass G3	Biomass G4	Biomass G5	Biomass G6	Biomass G7
Year	2004, 2012	1.711^{ns}	-	1.076^{ns}	-	0.343^{ns}	0.939^{ns}	0.133^{ns}	-
	2004, 2013	0.921^{ns}	-	2.301*	-	1.121^{ns}	1.433^{ns}	0.342^{ns}	-
	2005, 2006	1.557^{ns}	-	0.724^{ns}	-	2.475*	0.379^{ns}	0.753^{ns}	-
	2005, 2007	0.535^{ns}	-	0.568^{ns}	-	0.922^{ns}	0.789^{ns}	0.963^{ns}	-
	2005, 2008	0.715^{ns}	-	0.882^{ns}	-	0.616^{ns}	0.797^{ns}	0.892^{ns}	-
	2005, 2009	2.271*	-	0.441^{ns}	-	1.241^{ns}	1.401^{ns}	2.601*	-
	2005, 2010	0.922^{ns}	-	1.609^{ns}	-	1.845*	0.651^{ns}	0.128^{ns}	-
	2005, 2011	1.005^{ns}	-	0.904^{ns}	-	3.165*	1.468^{ns}	2.125^{ns}	-
	2005, 2012	1.864*	-	0.803^{ns}	-	1.515^{ns}	0.993^{ns}	1.685^{ns}	-
	2005, 2013	1.513^{ns}	-	1.923*	-	2.257*	2.644*	1.371^{ns}	-
	2006, 2007	0.919^{ns}	-	0.481^{ns}	-	1.698^{ns}	1.039^{ns}	1.551^{ns}	-
	2006, 2008	1.463^{ns}	-	0.906^{ns}	-	1.447^{ns}	0.989^{ns}	0.417^{ns}	-
	2006, 2009	2.787*	-	0.812^{ns}	-	0.901^{ns}	1.574^{ns}	1.784*	-
	2006, 2010	0.756^{ns}	-	2.085*	-	0.852^{ns}	0.903^{ns}	0.706^{ns}	-
	2006, 2011	1.068^{ns}	-	1.415^{ns}	-	0.434^{ns}	0.289^{ns}	1.306^{ns}	-
	2006, 2012	0.467^{ns}	-	0.957^{ns}	-	1.288^{ns}	0.578^{ns}	0.931^{ns}	-
	2006, 2013	0.620^{ns}	-	2.491*	-	0.984^{ns}	2.235*	0.623^{ns}	-
	2007, 2008	0.756^{ns}	-	0.741^{ns}	-	0.642^{ns}	0.601^{ns}	1.566^{ns}	-
	2007, 2009	2.194*	-	0.498^{ns}	-	0.651^{ns}	0.759^{ns}	3.473*	-
	2007, 2010	0.438^{ns}	-	1.647^{ns}	-	1.629^{ns}	0.269^{ns}	0.676^{ns}	-
	2007, 2011	0.711^{ns}	-	1.050^{ns}	-	2.287*	1.010^{ns}	2.837^{ns}	-
	2007, 2012	1.1725^{ns}	-	0.653^{ns}	-	0.608^{ns}	1.654^{ns}	0.710^{ns}	-
	2007, 2013	0.911^{ns}	-	2.066*	-	1.380^{ns}	2.914*	1.414^{ns}	-
	2008, 2009	1.082^{ns}	-	0.682^{ns}	-	0.882^{ns}	0.624^{ns}	1.451^{ns}	-
	2008, 2010	0.882^{ns}	-	0.953^{ns}	-	1.341^{ns}	0.450^{ns}	0.502^{ns}	-
	2008, 2011	1.421^{ns}	-	0.762^{ns}	-	1.87^{ns}	1.048^{ns}	1.157^{ns}	-
	2008, 2012	1.779*	-	0.475^{ns}	-	0.893^{ns}	1.438^{ns}	1.291^{ns}	-
	2008, 2013	1.254^{ns}	-	1.341^{ns}	-	1.601^{ns}	2.050^{ns}	0.539^{ns}	-
	2009, 2010	2.033*	-	1.206^{ns}	-	1.154^{ns}	0.813^{ns}	1.620^{ns}	-
	2009, 2011	2.931*	-	0.615^{ns}	-	1.301^{ns}	1.529^{ns}	3.103*	-
	2009, 2012	3.195*	-	0.397^{ns}	-	0.193^{ns}	2.182*	3.316*	-
	2009, 2013	2.338*	-	1.689^{ns}	-	0.631^{ns}	2.981*	2.249*	-
	2010, 2011	0.980^{ns}	-	0.649^{ns}	-	0.924^{ns}	0.948^{ns}	1.266^{ns}	-
	2010, 2012	1.002^{ns}	-	1.032^{ns}	-	1.449^{ns}	1.459^{ns}	1.614^{ns}	-
	2010, 2013	0.615^{ns}	-	1.079^{ns}	-	1.462^{ns}	2.579*	1.005^{ns}	-

Table 1 (continued).

Factor	Levels	Total Biomass	Biomass G1	Biomass G2	Biomass G3	Biomass G4	Biomass G5	Biomass G6	Biomass G7
Year	2011, 2012	1.157^{ns}	-	0.641^{ns}	-	1.826^{ns}	0.761^{ns}	0.618^{ns}	-
	2011, 2013	1.351^{ns}	-	1.116^{ns}	-	1.368^{ns}	2.413*	1.014^{ns}	-
	2012, 2013	0.825^{ns}	-	1.669^{ns}	-	0.844^{ns}	2.103*	0.974^{ns}	-

Table 2 - PERMANOVA results from the pair-wise tests for the differences in density of each functional group considering factors season, station and year. Each value corresponds to the t statistic and the significance is in superscript (* p-value < 0.05, ns - non-significant). Blank cells correspond to non-significant main tests.

Factor	Levels	Total Density	Density G1	Density G2	Density G3	Density G4	Density G5	Density G6	Density G7
Season	summer, autumn	1.968*	-	-	-	-	-	-	-
	summer, winter	3.198*	-	-	-	-	-	-	-
	summer, spring	2.069*	-	-	-	-	-	-	-
	autumn, winter	1.752^{ns}	-	-	-	-	-	-	-
	autumn, spring	0.209^{ns}	-	-	-	-	-	-	-
	winter, spring	1.803^{ns}	-	-	-	-	-	-	-
Station	M, S1	6.314*	1.447^{ns}	1.292^{ns}	0.438^{ns}	0.676^{ns}	6.795*	2.275*	-
	M, S2	3.668*	0.678^{ns}	1.077^{ns}	0.789^{ns}	4.676*	3.113*	7.600*	-
	M, N1	0.701^{ns}	3.098*	1.925*	2.735*	1.947^{ns}	3.226*	3.035*	-
	M, N2	0.990^{ns}	1.584^{ns}	2.156*	0.971^{ns}	1.162^{ns}	4.601*	4.029*	-
	S1, S2	10.769*	1.185^{ns}	2.021*	0.589^{ns}	3.514*	3.829*	1.039*	-
	S1, N1	6.663*	1.887^{ns}	1.204^{ns}	2.531*	2.998*	3.587*	5.519*	-
	S1, N2	4.996*	1.665^{ns}	2.171*	0.989^{ns}	1.905^{ns}	1.592^{ns}	6.470*	-
	S2, N1	4.363*	3.131*	2.844*	2.752*	4.938*	0.442^{ns}	4.727*	-
	S2, N2	4.514*	1.925*	3.095*	1.605^{ns}	2.983*	1.929*	3.334*	-
	N1, N2	1.186^{ns}	0.830^{ns}	1.158^{ns}	0.311^{ns}	0.228^{ns}	1.725^{ns}	1.230^{ns}	-
Year	2003, 2004	1.631^{ns}	0.413^{ns}	1.779^{ns}	1.098^{ns}	-	1.223^{ns}	1.060^{ns}	-
	2003, 2005	3.163*	0.354^{ns}	2.037*	2.413*	-	2.643*	2.429*	-
	2003, 2006	2.471*	1.724^{ns}	1.547^{ns}	0.710^{ns}	-	0.761^{ns}	1.636^{ns}	-
	2003, 2007	3.313*	1.610^{ns}	1.972*	0.394^{ns}	-	1.309^{ns}	2.923*	-
	2003, 2008	1.982*	0.956^{ns}	2.422*	0.609^{ns}	-	0.986^{ns}	1.063^{ns}	-
	2003, 2009	1.973*	0.759^{ns}	1.682^{ns}	1.012^{ns}	-	0.641^{ns}	1.623*	-
	2003, 2010	2.165*	0.674^{ns}	0.906^{ns}	0.393^{ns}	-	0.499^{ns}	1.980*	-
	2003, 2011	0.869^{ns}	1.792^{ns}	1.892^{ns}	0.626^{ns}	-	1.433^{ns}	0.872^{ns}	-
	2003, 2012	1.536^{ns}	0.668^{ns}	3.123*	0.912^{ns}	-	0.596^{ns}	0.964^{ns}	-
	2003, 2013	1.906*	0.394^{ns}	2.106*	0.352^{ns}	-	0.701^{ns}	1.226^{ns}	-
	2004, 2005	1.545^{ns}	0.201^{ns}	0.579^{ns}	1.145^{ns}	-	1.118^{ns}	1.793^{ns}	-
	2004, 2006	1.191^{ns}	2.254*	0.296^{ns}	0.458^{ns}	-	0.497^{ns}	0.977^{ns}	-
	2004, 2007	1.888*	1.263^{ns}	0.412^{ns}	1.434^{ns}	-	0.906^{ns}	2.542*	-
	2004, 2008	0.720^{ns}	0.732^{ns}	1.767^{ns}	0.524^{ns}	-	0.483^{ns}	0.528^{ns}	-
	2004, 2009	1.155^{ns}	1.058^{ns}	0.255^{ns}	0.293^{ns}	-	1.581^{ns}	0.959^{ns}	-
	2004, 2010	1.192^{ns}	0.305^{ns}	0.858^{ns}	0.789^{ns}	-	1.525^{ns}	1.542^{ns}	-
	2004, 2011	1.484^{ns}	2.328*	0.778^{ns}	1.702^{ns}	-	2.538*	0.518^{ns}	-
	2004, 2012	0.242^{ns}	0.452^{ns}	1.923^{ns}	1.855^{ns}	-	1.799^{ns}	0.133^{ns}	-
	2004, 2013	0.463^{ns}	0.725^{ns}	0.624^{ns}	1.370^{ns}	-	0.891^{ns}	0.342^{ns}	-
	2005, 2006	0.528^{ns}	2.039*	0.476^{ns}	1.705^{ns}	-	1.717^{ns}	0.753^{ns}	-

Table 2 (continued)

Factor	Levels	Total Density	Density G1	Density G2	Density G3	Density G4	Density G5	Density G6	Density G7
Year	2005, 2007	0.602^{ns}	1.352^{ns}	0.494^{ns}	2.765*	-	1.797^{ns}	0.963^{ns}	-
	2005, 2008	0.649^{ns}	0.756^{ns}	1.209^{ns}	1.667^{ns}	-	1.265^{ns}	0.892^{ns}	-
	2005, 2009	2.569*	1.074^{ns}	0.323^{ns}	1.111^{ns}	-	3.046*	2.601*	-
	2005, 2010	0.740^{ns}	0.475^{ns}	1.215^{ns}	1.972^{ns}	-	2.914*	0.128^{ns}	-
	2005, 2011	3.105*	2.278*	0.329^{ns}	3.119*	-	4.294*	2.125*	-
	2005, 2012	1.379^{ns}	0.435^{ns}	1.269^{ns}	3.200*	-	3.373*	1.685^{ns}	-
	2005, 2013	1.437^{ns}	0.680^{ns}	0.175^{ns}	2.691*	-	2.186*	1.371^{ns}	-
	2006, 2007	0.981^{ns}	3.622*	0.562^{ns}	1.089^{ns}	-	0.763^{ns}	1.551^{ns}	-
	2006, 2008	0.694^{ns}	2.433*	1.573^{ns}	0.116^{ns}	-	0.420^{ns}	0.417^{ns}	-
	2006, 2009	2.281*	2.505*	0.232^{ns}	0.537^{ns}	-	1.068^{ns}	1.783^{ns}	-
	2006, 2010	0.273^{ns}	2.931*	0.780^{ns}	0.388^{ns}	-	1.145^{ns}	0.707^{ns}	-
	2006, 2011	2.546*	0.825^{ns}	0.638^{ns}	1.349^{ns}	-	2.146*	1.306^{ns}	-
	2006, 2012	0.987^{ns}	2.100*	1.716^{ns}	1.559^{ns}	-	1.288^{ns}	0.931^{ns}	-
	2006, 2013	1.245^{ns}	1.377^{ns}	0.572^{ns}	0.994^{ns}	-	0.409^{ns}	0.623^{ns}	-
	2007, 2008	0.974^{ns}	0.371^{ns}	1.721^{ns}	0.991^{ns}	-	0.305^{ns}	1.566^{ns}	-
	2007, 2009	2.921*	2.733*	0.408^{ns}	1.283^{ns}	-	1.299^{ns}	3.473*	-
	2007, 2010	1.094^{ns}	1.289^{ns}	1.180^{ns}	0.776^{ns}	-	1.727^{ns}	0.676^{ns}	-
	2007, 2011	3.405*	4.049*	0.721^{ns}	0.234^{ns}	-	2.815*	2.837*	-
	2007, 2012	1.739^{ns}	0.767^{ns}	1.645^{ns}	0.559^{ns}	-	1.691^{ns}	2.411*	-
	2007, 2013	1.774^{ns}	1.831^{ns}	0.500^{ns}	0.343^{ns}	-	0.684^{ns}	2.141*	-
	2008, 2009	1.449^{ns}	1.799^{ns}	1.457^{ns}	0.584^{ns}	-	1.127^{ns}	1.288*	-
	2008, 2010	0.857^{ns}	0.813^{ns}	2.117*	0.278^{ns}	-	1.339^{ns}	0.846^{ns}	-
	2008, 2011	1.941^{ns}	2.726*	1.035^{ns}	1.261^{ns}	-	2.209*	0.894^{ns}	-
	2008, 2012	0.715^{ns}	0.314^{ns}	0.502^{ns}	1.536^{ns}	-	1.398^{ns}	0.467^{ns}	-
	2008, 2013	0.559^{ns}	1.145^{ns}	1.192^{ns}	0.898^{ns}	-	0.498^{ns}	0.359^{ns}	-
	2009, 2010	2.285*	1.328^{ns}	0.887^{ns}	0.779^{ns}	-	0.919^{ns}	2.338*	-
	2009, 2011	1.382^{ns}	1.897^{ns}	0.500^{ns}	1.516^{ns}	-	1.812^{ns}	0.816^{ns}	-
	2009, 2012	1.348^{ns}	1.413^{ns}	1.553^{ns}	1.625^{ns}	-	0.464^{ns}	0.997^{ns}	-
	2009, 2013	1.001^{ns}	0.495^{ns}	0.362^{ns}	1.252^{ns}	-	0.764^{ns}	1.125^{ns}	-
	2010, 2011	2.365*	2.801*	1.198^{ns}	1.029^{ns}	-	0.812^{ns}	1.846*	-
	2010, 2012	0.971^{ns}	0.585^{ns}	2.414*	1.352^{ns}	-	0.620^{ns}	1.461^{ns}	-
	2010, 2013	1.321^{ns}	0.973^{ns}	1.259^{ns}	0.639^{ns}	-	1.159^{ns}	1.221^{ns}	-
	2011, 2012	1.515^{ns}	2.386*	1.026^{ns}	0.399^{ns}	-	1.373^{ns}	0.547^{ns}	-
	2011, 2013	1.629^{ns}	1.104^{ns}	0.296^{ns}	0.474^{ns}	-	2.219*	0.726^{ns}	-
	2012, 2013	0.611^{ns}	0.888^{ns}	1.251^{ns}	0.377^{ns}	-	1.091^{ns}	0.360^{ns}	-

Table 3 – PERMANOVA results from the pair-wise tests for the differences in number of species (Num. Sp.) of each functional group considering factors season, station and year. Each value corresponds to the t statistic and the significance is in superscript (* p-value < 0.05, ns - non-significant). Blank cells correspond to non-significant main tests.

Factor	Levels	Total Num. Sp.	Num. Sp. G1	Num. Sp. G2	Num. Sp. G3	Num. Sp. G4	Num. Sp. G5	Num. Sp. G6	Num. Sp. G7
Season	summer, autumn	-	-	-	2.863*	0.113^{ns}	0.800^{ns}	-	-
	summer, winter	-	-	-	3.555*	2.180^{ns}	3.150*	-	-
	summer, spring	-	-	-	1.453^{ns}	1.282^{ns}	0.382^{ns}	-	-
	autumn, winter	-	-	-	1.111^{ns}	2.042^{ns}	2.663*	-	-
	autumn, spring	-	-	-	1.064^{ns}	1.173^{ns}	1.165^{ns}	-	-
	winter, spring	-	-	-	1.765^{ns}	3.314*	3.331*	-	-
Station	M, S1	9.436*	2.320*	2.225*	2.135*	-	8.799*	6.506*	-
	M, S2	4.867*	3.294*	4.003*	2.403*	-	5.860*	1.894^{ns}	-
	M, N1	3.743*	1.630^{ns}	1.241^{ns}	3.342*	-	4.312*	2.826*	-
	M, N2	8.322*	0.618^{ns}	2.346*	1.094^{ns}	-	11.346*	3.961*	-
	S1, S2	6.904*	1.220^{ns}	2.234*	0.942^{ns}	-	2.738*	10.223*	-
	S1, N1	6.806*	1.199^{ns}	1.114^{ns}	1.260^{ns}	-	4.907*	10.057*	-
	S1, N2	1.499^{ns}	0.223^{ns}	1.300^{ns}	0.629^{ns}	-	1.707^{ns}	10.72*	-
	S2, N1	0.951^{ns}	2.379*	2.962*	0.038^{ns}	-	1.908^{ns}	1.327^{ns}	-
	S2, N2	5.422*	-	-	0.521^{ns}	-	4.623*	2.741*	-
	N1, N2	5.509*	0.439^{ns}	1.732^{ns}	0.420^{ns}	-	7.301*	1.361^{ns}	-
Year	2003, 2004	-	1.022^{ns}	-	-	-	1.293^{ns}	0.697^{ns}	-
	2003, 2005	-	1.776^{ns}	-	-	-	1.384^{ns}	2.149*	-
	2003, 2006	-	0.228^{ns}	-	-	-	0.652^{ns}	2.493*	-
	2003, 2007	-	0.369^{ns}	-	-	-	1.977^{ns}	2.601*	-
	2003, 2008	-	1^{ns}	-	-	-	1.283^{ns}	1.126^{ns}	-
	2003, 2009	-	2.837*	-	-	-	0.237^{ns}	0.297^{ns}	-
	2003, 2010	-	1.030^{ns}	-	-	-	0.734^{ns}	1.531^{ns}	-
	2003, 2011	-	3.159*	-	-	-	1.382^{ns}	0.797^{ns}	-
	2003, 2012	-	1.517^{ns}	-	-	-	0.576^{ns}	2.301*	-
	2003, 2013	-	1.902^{ns}	-	-	-	0.467^{ns}	0.753^{ns}	-
	2004, 2005	-	2.569*	-	-	-	0.103^{ns}	1.516^{ns}	-
	2004, 2006	-	0.824^{ns}	-	-	-	0.612^{ns}	1.976^{ns}	-
	2004, 2007	-	0.409^{ns}	-	-	-	0.446^{ns}	2.116*	-
	2004, 2008	-	1.545^{ns}	-	-	-	0.022^{ns}	0.653^{ns}	-
	2004, 2009	-	3.417*	-	-	-	1.125^{ns}	1.119^{ns}	-
	2004, 2010	-	1.756^{ns}	-	-	-	0.539^{ns}	1.058^{ns}	-
	2004, 2011	-	3.810*	-	-	-	2.553*	0.160^{ns}	-
	2004, 2012	-	2.127^{ns}	-	-	-	1.867^{ns}	1.794^{ns}	-

Table 3 (continued)

Factor	Levels	Total Num. Sp.	Num. Sp. G1	Num. Sp. G2	Num. Sp. G3	Num. Sp. G4	Num. Sp. G5	Num. Sp. G6	Num. Sp. G7
Year	2004, 2013	-	2.278^{ns}	-	-	-	1.846^{ns}	0.051^{ns}	-
	2005, 2006	-	0.875^{ns}	-	-	-	0.609^{ns}	0.591^{ns}	-
	2005, 2007	-	1.637^{ns}	-	-	-	0.621^{ns}	0.703^{ns}	-
	2005, 2008	-	0.292^{ns}	-	-	-	0.116^{ns}	0.474^{ns}	-
	2005, 2009	-	0.823^{ns}	-	-	-	1.181^{ns}	2.74*	-
	2005, 2010	-	0.543^{ns}	-	-	-	0.517^{ns}	0.975^{ns}	-
	2005, 2011	-	0.915^{ns}	-	-	-	2.842*	1.243^{ns}	-
	2005, 2012	-	0.034^{ns}	-	-	-	2.039*	0.433^{ns}	-
	2005, 2013	-	0.554^{ns}	-	-	-	1.989^{ns}	1.474^{ns}	-
	2006, 2007	-	0.385^{ns}	-	-	-	1.116^{ns}	0.109^{ns}	-
	2006, 2008	-	0.447^{ns}	-	-	-	0.575^{ns}	0.894^{ns}	-
	2006, 2009	-	1.525^{ns}	-	-	-	0.457^{ns}	3.187*	-
	2006, 2010	-	0.435^{ns}	-	-	-	0.062^{ns}	0.543^{ns}	-
	2006, 2011	-	1.706^{ns}	-	-	-	1.930^{ns}	1.698^{ns}	-
	2006, 2012	-	0.739^{ns}	-	-	-	1.207^{ns}	0.133^{ns}	-
	2006, 2013	-	1^{ns}	-	-	-	1.136^{ns}	1.988^{ns}	-
	2007, 2008	-	0.892^{ns}	-	-	-	0.452^{ns}	1.024^{ns}	-
	2007, 2009	-	2.137^{ns}	-	-	-	1.849^{ns}	3.409*	-
	2007, 2010	-	1^{ns}	-	-	-	1.134^{ns}	0.641^{ns}	-
	2007, 2011	-	2.383*	-	-	-	3.411*	1.847^{ns}	-
	2007, 2012	-	1.283^{ns}	-	-	-	2.684*	0.247^{ns}	-
	2007, 2013	-	1.421^{ns}	-	-	-	2.844*	2.189*	-
	2008, 2009	-	1.525^{ns}	-	-	-	1.168^{ns}	1.557^{ns}	-
	2008, 2010	-	0.154^{ns}	-	-	-	0.594^{ns}	0.295^{ns}	-
	2008, 2011	-	1.705^{ns}	-	-	-	2.504*	0.493^{ns}	-
	2008, 2012	-	0.289^{ns}	-	-	-	1.879^{ns}	0.776^{ns}	-
	2008, 2013	-	1^{ns}	-	-	-	2.037^{ns}	0.646^{ns}	-
	2009, 2010	-	1.722^{ns}	-	-	-	0.551^{ns}	2.008^{ns}	-
	2009, 2011	-	-	-	-	-	1.682^{ns}	1.224^{ns}	-
	2009, 2012	-	1.215^{ns}	-	-	-	0.853^{ns}	2.989*	-
	2009, 2013	-	-	-	-	-	0.770^{ns}	1.218^{ns}	-
	2010, 2011	-	1.921^{ns}	-	-	-	2.035^{ns}	0.863^{ns}	-
	2010, 2012	-	0.505^{ns}	-	-	-	1.318^{ns}	0.423^{ns}	-
	2010, 2013	-	1.145^{ns}	-	-	-	1.317^{ns}	1.045^{ns}	-
	2011, 2012	-	1.356^{ns}	-	-	-	0.868^{ns}	1.538^{ns}	-
	2011, 2013	-	-	-	-	-	1.102^{ns}	0.118^{ns}	-
	2012, 2013	-	0.806^{ns}	-	-	-	0.168^{ns}	1.820^{ns}	-

Appendix III - Significant environmental variables and factors in GLM - visualization of regression plots (Stations: 2-M; 5-S1; 9-S2; 12-N1; 19-N2)

Biomass

Mobility

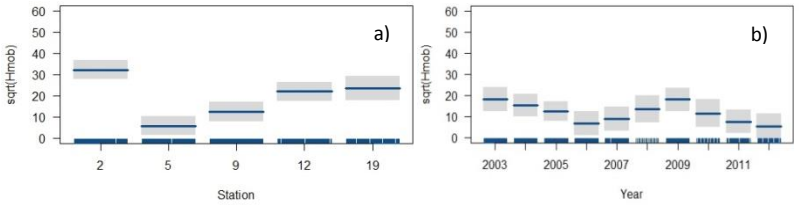


Figure 1 – Response of high mobility to factors station (a) and year (b)

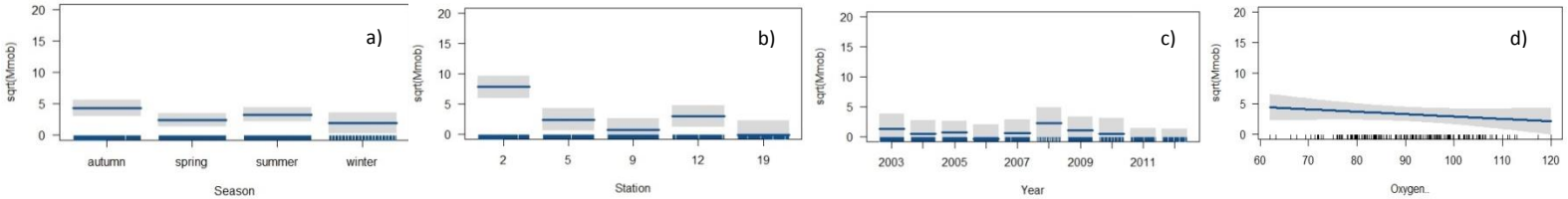


Figure 2 – Response of medium mobility to factors season (a), station (b) and year (c) and to variable oxygen (d)

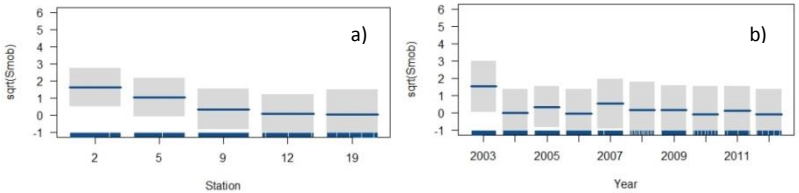


Figure 3 – Response of sedentary mobility to factors station (a) and year (b)

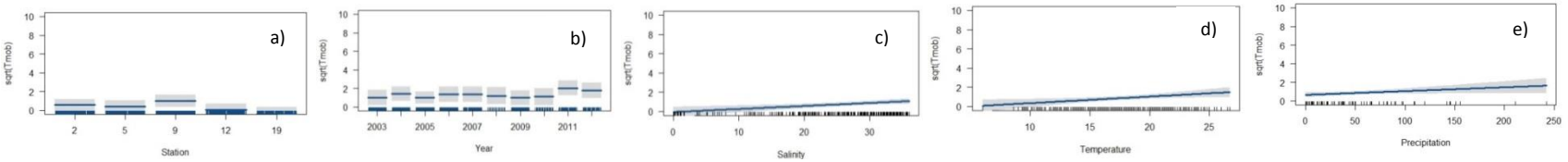


Figure 4 – Response of territorial mobility to factors station (a) and year (b) and to variables salinity (c), temperature (d) and precipitation (e)

Body size

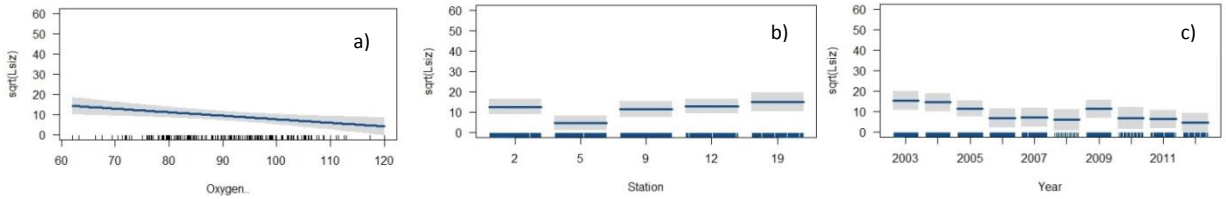


Figure 5 – Response of large body size to variable oxygen (a) and to factors station (b) and year (c)

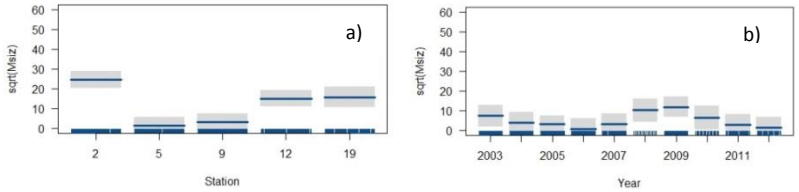


Figure 6 – Response of medium body size to factors station (a) and year (b)

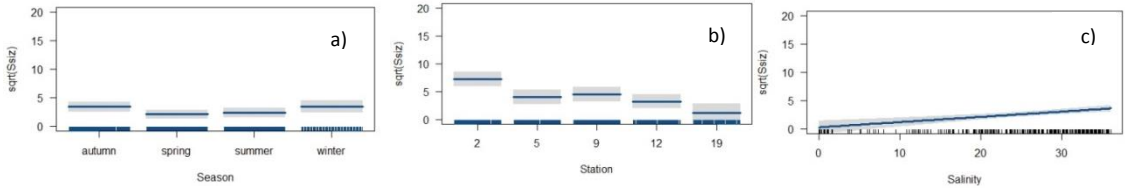


Figure 7 – Response of small body size to factors season (a) and station (b) and to variable salinity (c)

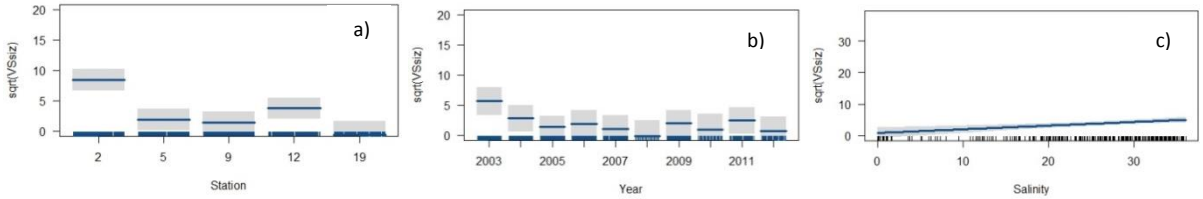


Figure 8– Response of very small body size to factors station (a) and year (b) and to variable salinity (c)

Diet

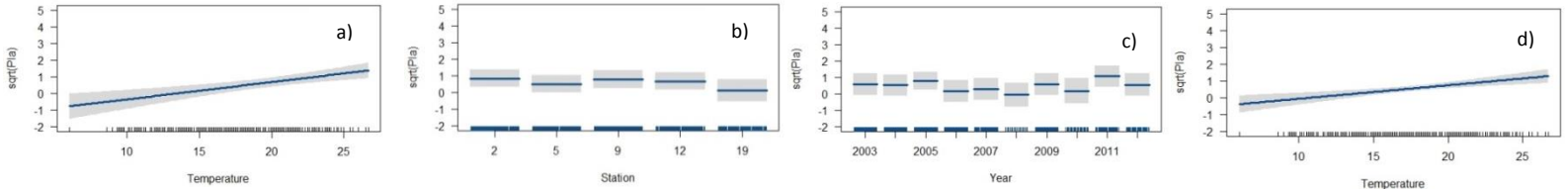


Figure 9 – Response of planktivorous to temperature (GLM with factors) (a), to factors station (b) and year (c) and to variable temperature (GLM without factors) (d)

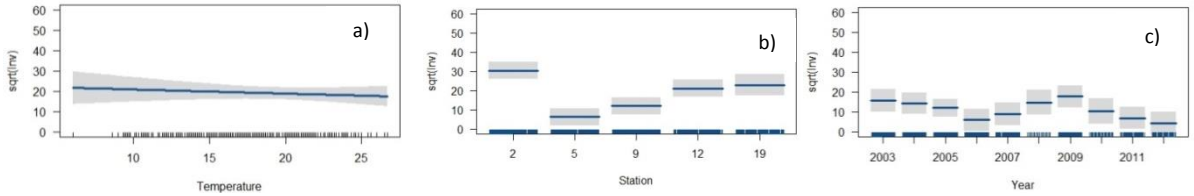


Figure 10 – Response of invertebrate feeders to variable temperature (a) and to factors station (b) and year (c)

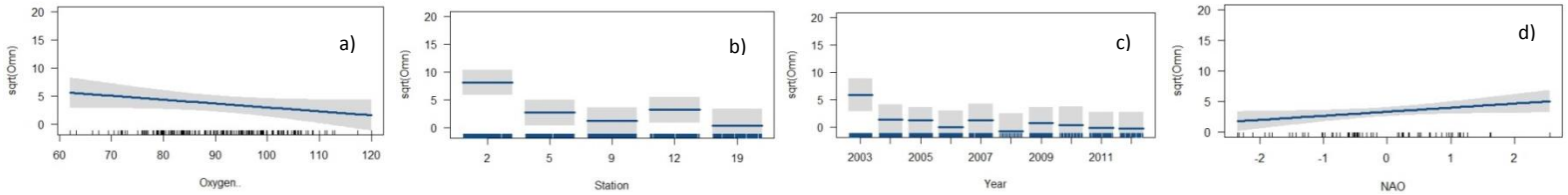


Figure 11 – Response of omnivorous to variable oxygen (a), to factors station (b) and year (c) and to variable NAO index (d)

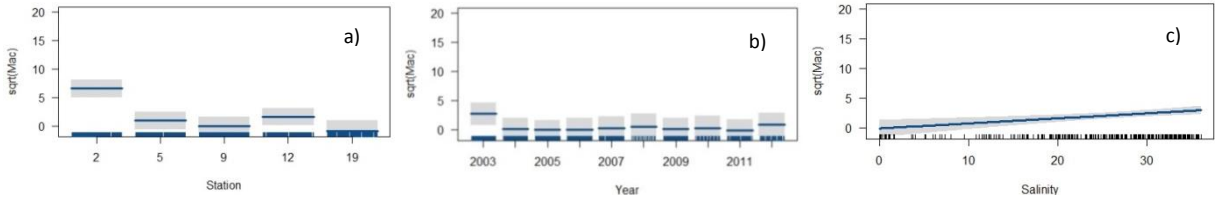


Figure 12 – Response of macrocarnivorous to factors station (a) and year (b) and to variable salinity (c)

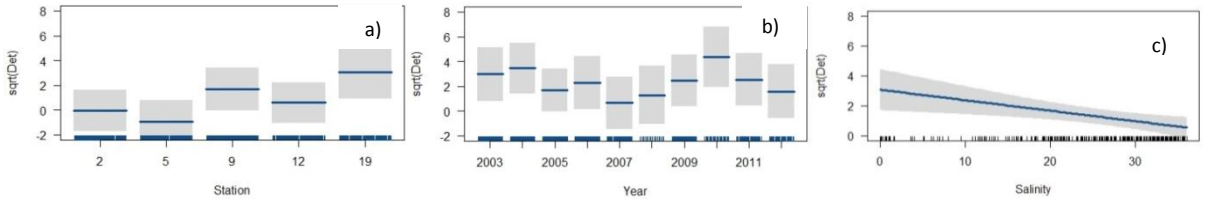


Figure 13 – Response of detritivorous to factors station (a) and year (b) and to variable salinity (c)

Feeding mode

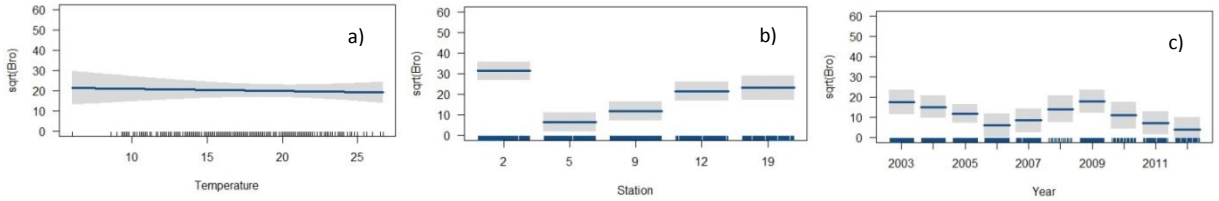


Figure 14 – Response of browsers to variable temperature (a) and to factors station (b) and year (c)

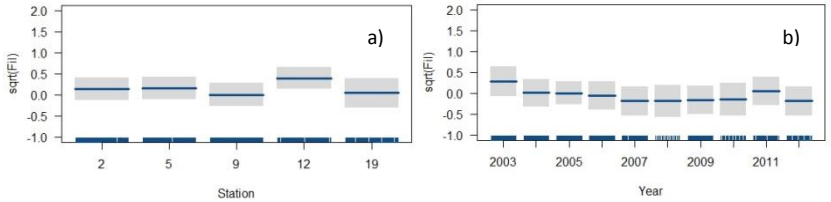


Figure 15– Response of filterers to factors station (a) and year (b)

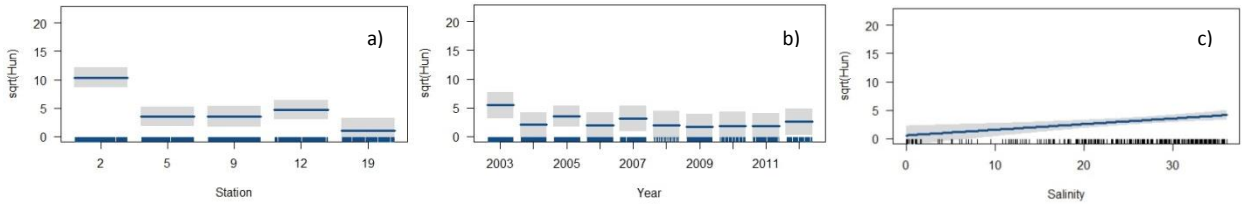


Figure 16 – Response of hunters to factors station (a) and year (b) and to variable salinity (c)

Salinity preference

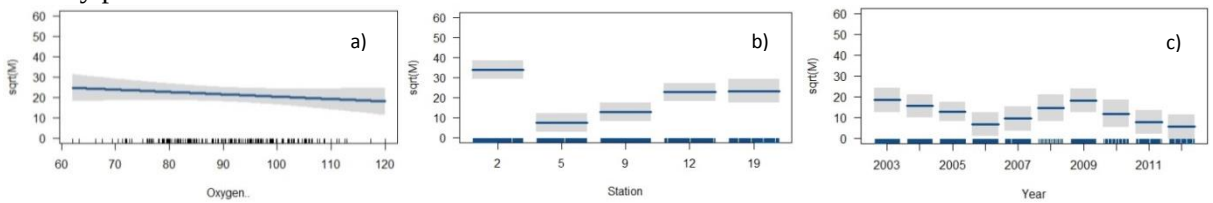


Figure 17– Response of marine species to variable oxygen (a) and to factors station (b) and year (c)

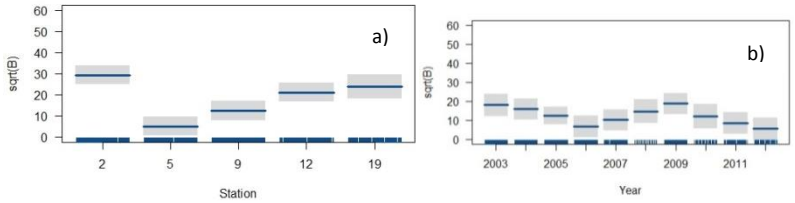


Figure 18– Response of brackish species to factors station (a) and year (b)

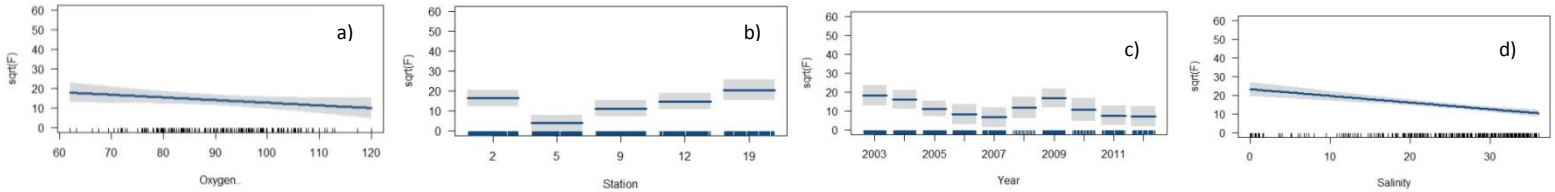


Figure 19 – Response of freshwater species to variable oxygen (a), to factors station (b) and year (c) and to variable salinity (d)

Density

Mobility

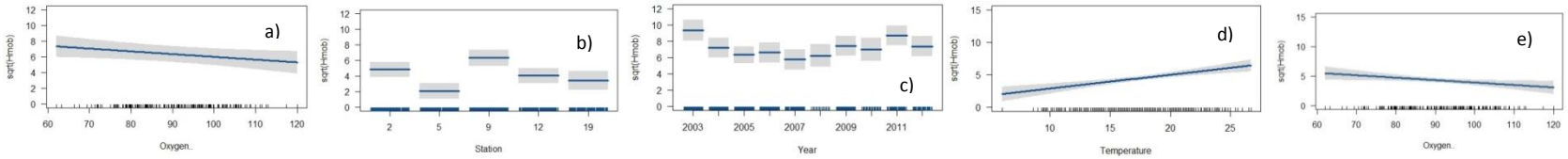


Figure 20 – Response of high mobility to variable oxygen (GLM with factors) (a), to factors station (b) and year (c) and to variables temperature (d) and oxygen (GLM without factors) (e)

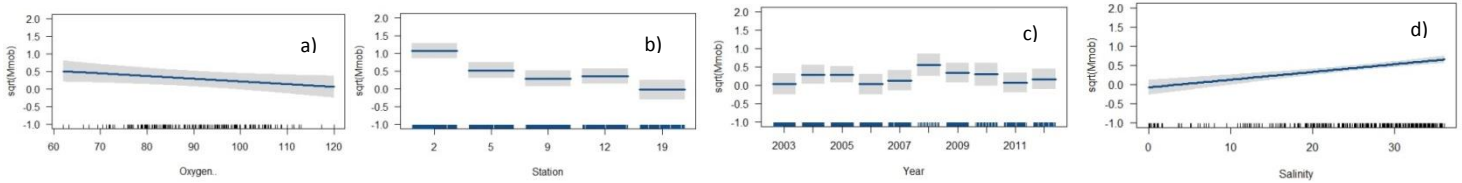


Figure 21– Response of medium mobility to variable oxygen (a), to factors station (b) and year (c) and to variable salinity (d)

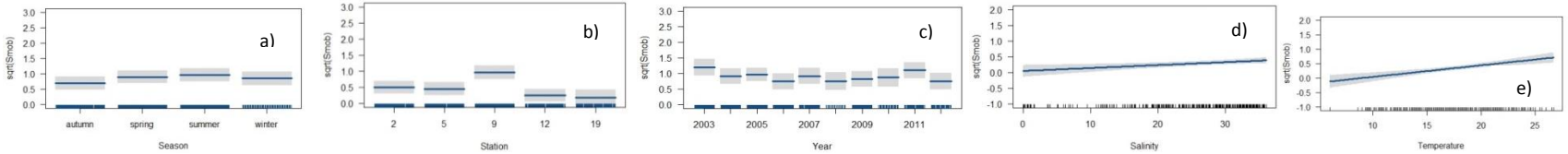


Figure 22 – Response of sedentary mobility to factors season (a), station (b) and year (c) and to variables salinity (d) and temperature (e)

Body size

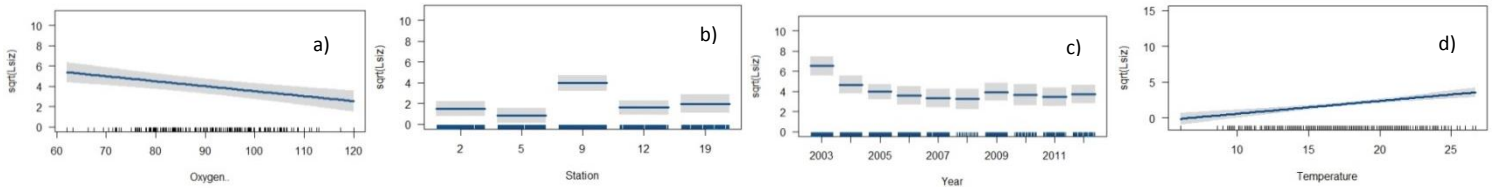


Figure 23 – Response of large body size to variable oxygen (GLM with factors) (a), to factors station (b) and year (c) and to variable temperature (d)

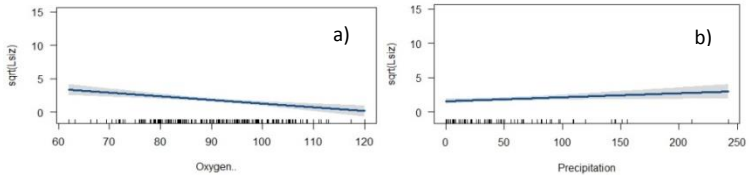


Figure 24– Response of large body size to variable oxygen (GLM without factors) (a) and precipitation (b)

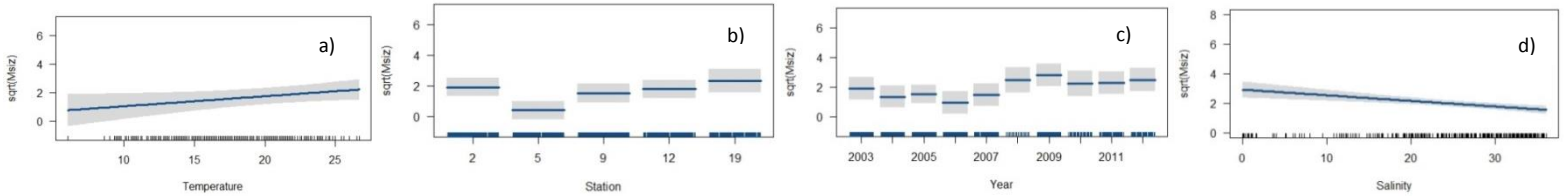


Figure 25 – Response of medium body size to variable temperature (a), to factors station (b) and year (c) and to variable salinity (d)

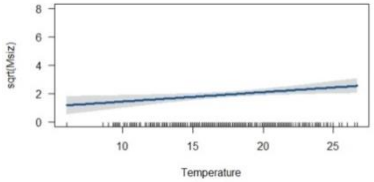


Figure 26 – Response of medium body size to variable temperature (GLM without factors)

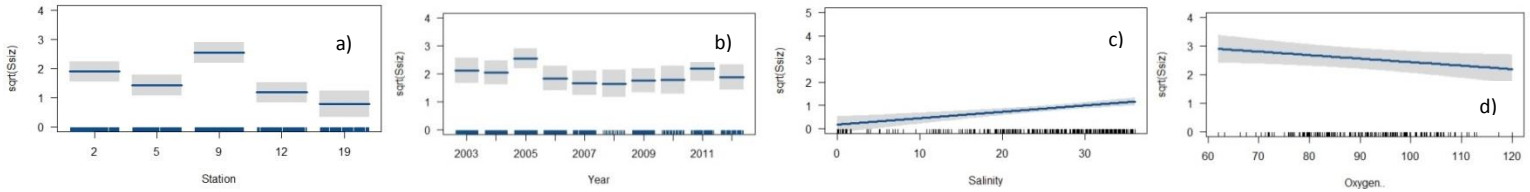


Figure 27 – Response of small body size to factors station (a) and year (b) and to variables salinity (d) and oxygen (d)

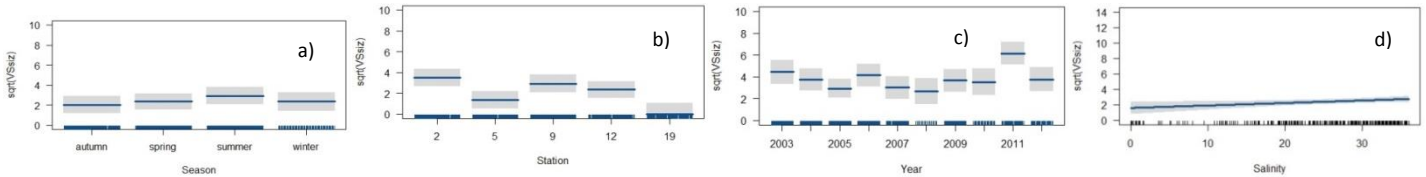


Figure 28 – Response of very small body size to factor season (a), station (b) and year (c) and to variable salinity (d)

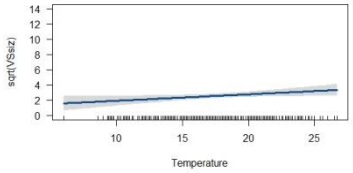


Figure 29 – Response of very small body size to variable temperature

Diet

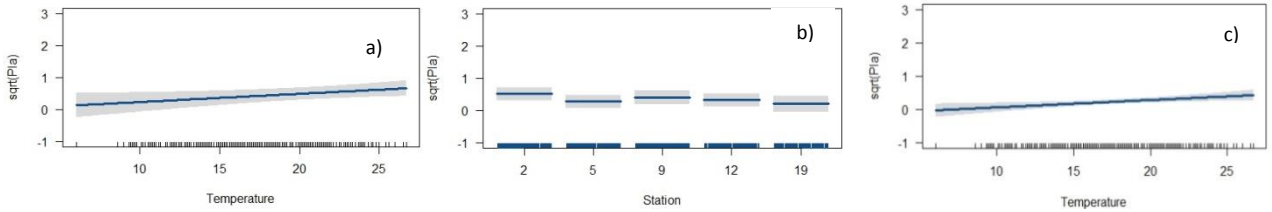


Figure 30 – Response of planktivorous to variable temperature (GLM with factors) (a), to factors station (b) and to variable temperature (GLM without factors) (c)

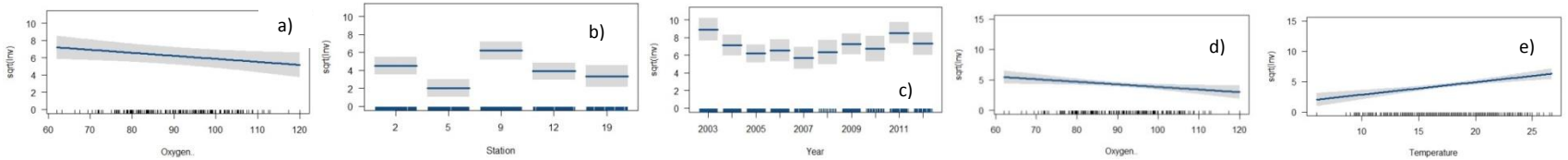


Figure 31 – Response of invertebrate feeders to oxygen (GLM with factors) (a), to factors station (b) and year (c) and to variables oxygen (GLM without factors) (d) and temperature (e)

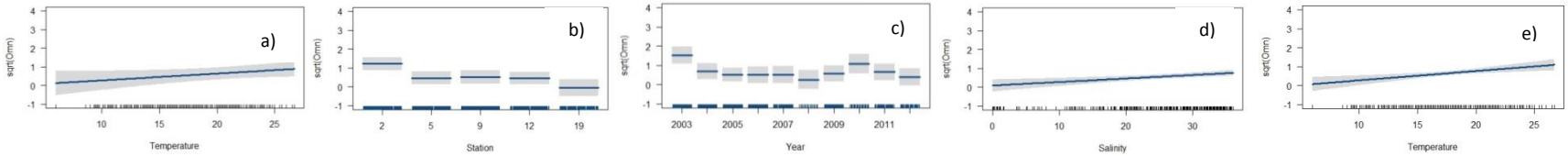
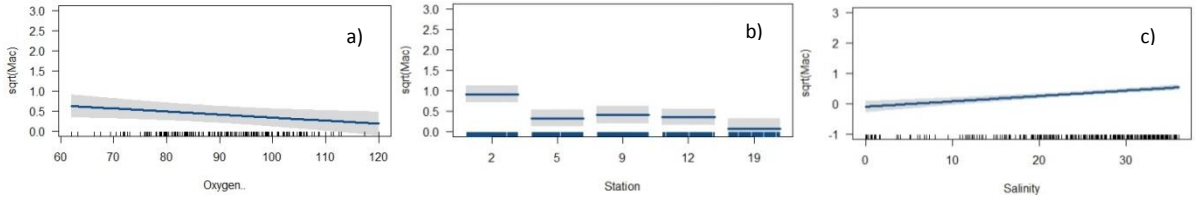


Figure 32 – Response of omnivorous to variable temperature (GLM with factors) (a), to factors station (b) and year (c) and to variables salinity (d) and temperature (GLM without factors) (e)



Figures 33 – Response of macrocarnivorous to variable oxygen (a), to factor station (b) and to variable salinity (c)

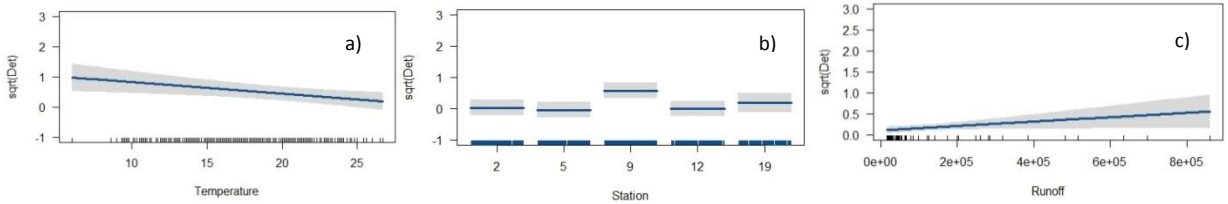


Figure 34 – Response of detritivorous to variable temperature (a), to factor station (b) and to variable runoff (c)

Feeding mode

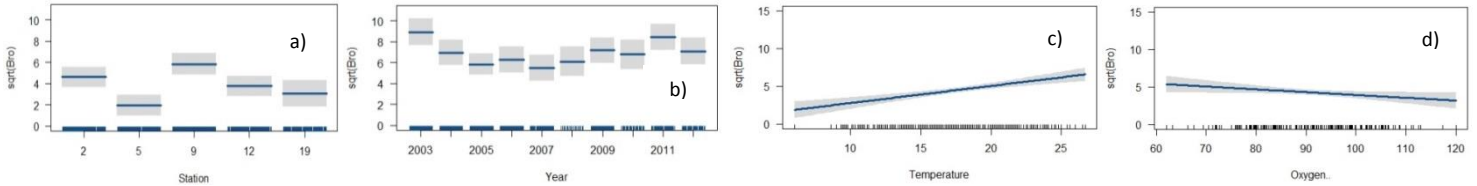


Figure 35 – Response of browsers to factors station (a) and year (b) and to variables temperature (c) and oxygen (d)

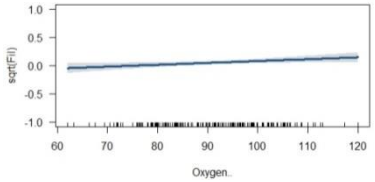


Figure 36 – Response of filterers to variable oxygen

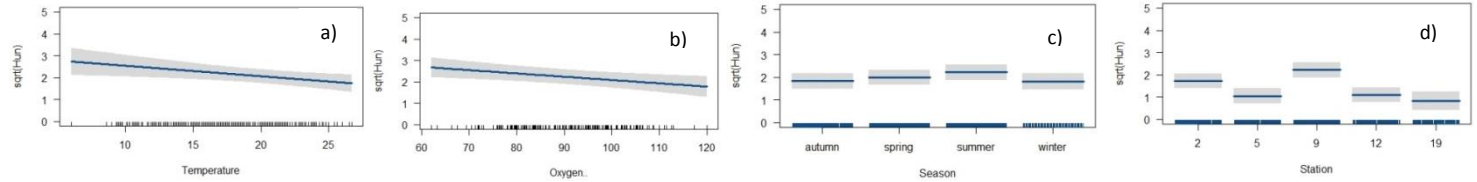


Figure 37 – Response of hunters to variables temperature (a) and oxygen (b) and to factors season (c) and station (d)

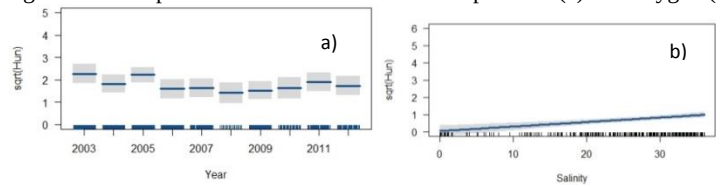


Figure 38 – Response of hunters to factor year (a) and to variable salinity (b)

Salinity preference

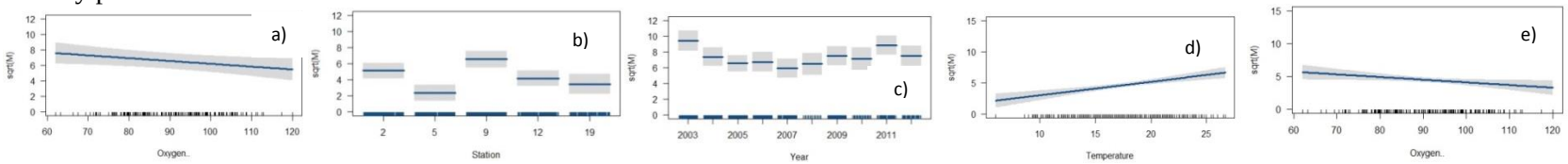


Figure 39– Response of marine species to variable oxygen (GLM with factors) (a), to factors station (b) and year (c) and to variables temperature (d) and oxygen (GLM without factors) (e)

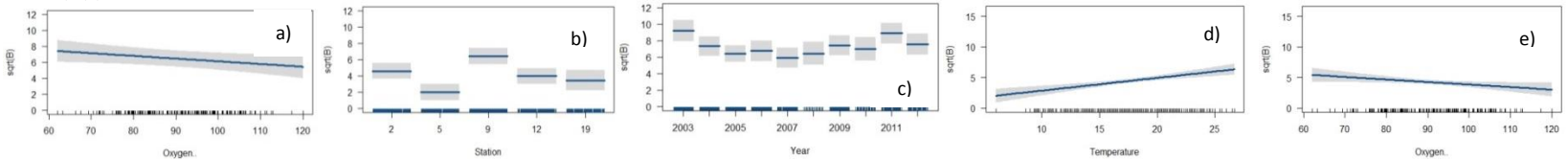


Figure 40 – Response of brackish species to variable oxygen (GLM with factors) (a), to factors station (b) and year (c) and to variables temperature (d) and oxygen (GLM without factors) (e)

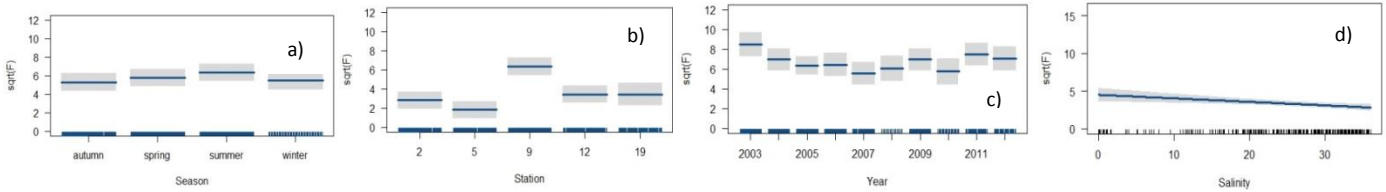
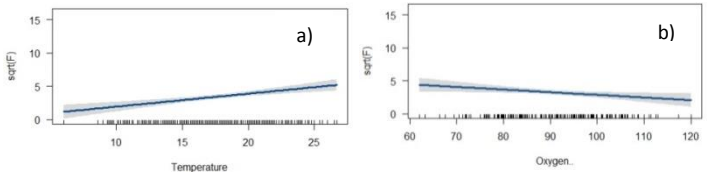


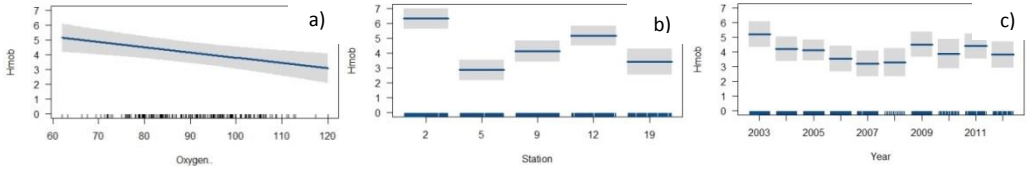
Figure 41 – Response of freshwater species to factors season (a) station (b) and year (c) and to variable salinity (d)



Figures 42 – Response of freshwater species to variables temperature (a) and oxygen (b)

Number of species

Mobility



Figures 43 – Response of high mobility to variable oxygen (a) and to factors station (b) and year (c)

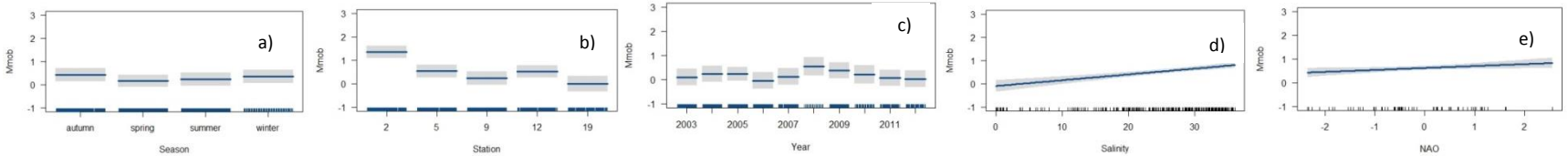


Figure 44 – Response of medium mobility to factors season (a), station (b) and year (c) and to variables salinity (d) and NAO index (e)

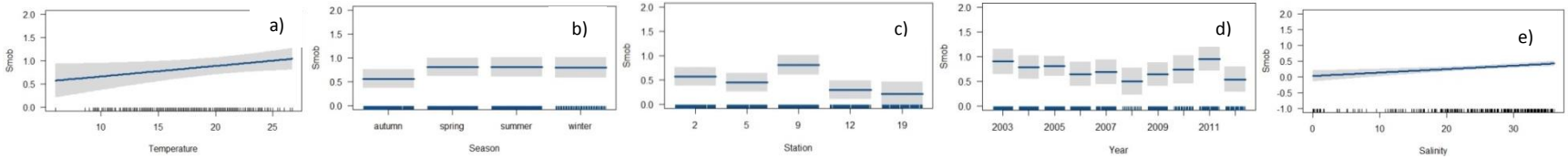


Figure 45 – Response of sedentary mobility to variable temperature (GLM with factors) (a), to factors season (b), station (c) and year (d) and to variable salinity (e)

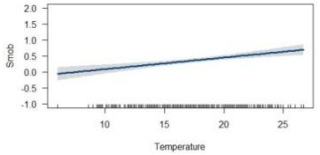


Figure 46 – Response of sedentary mobility to variable temperature (GLM without factors)

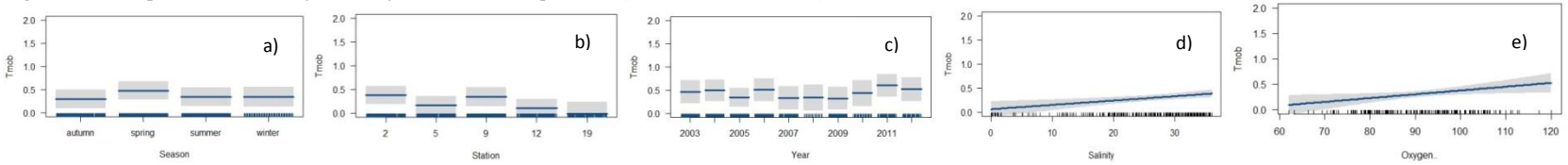


Figure 47 – Response of territorial mobility to factors season (a), station (b) and year (c) and to variables salinity (d) and oxygen (e)

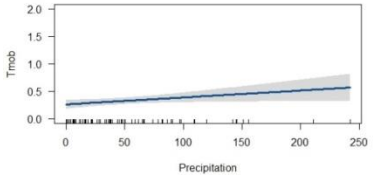


Figure 48 – Response of territorial mobility to variable precipitation

Body size

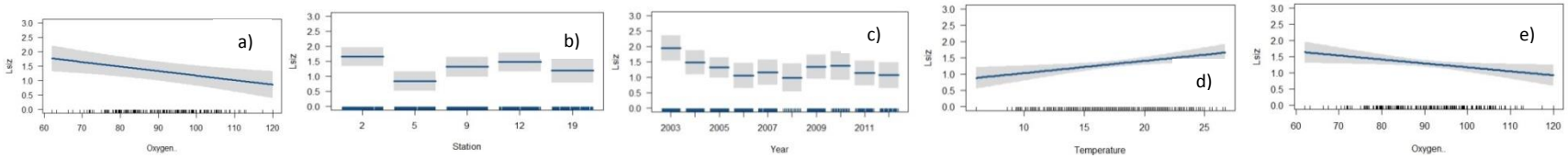


Figure 49 – Response of large body size to oxygen (GLM with factors) (a), to factors station (b) and year (c) and to variables temperature (d) and oxygen (GLM without factors) (e)

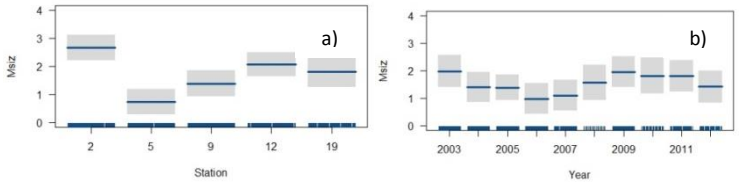


Figure 50 – Response of medium body size to factors station (a) and year (b)

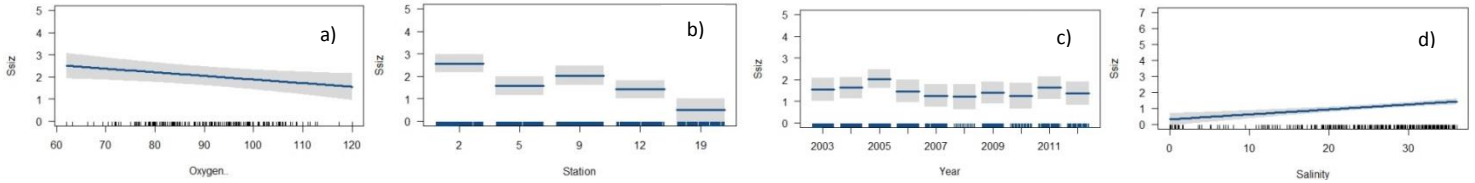


Figure 51 – Response of small body size to variable oxygen (a), to factors station (b) and year (c) and to variable salinity (d)

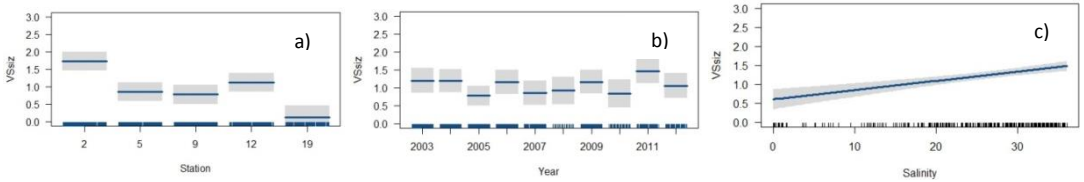


Figure 52 – Response of very small body size to factors station (a) and year (b) and to variable salinity (c)

Diet

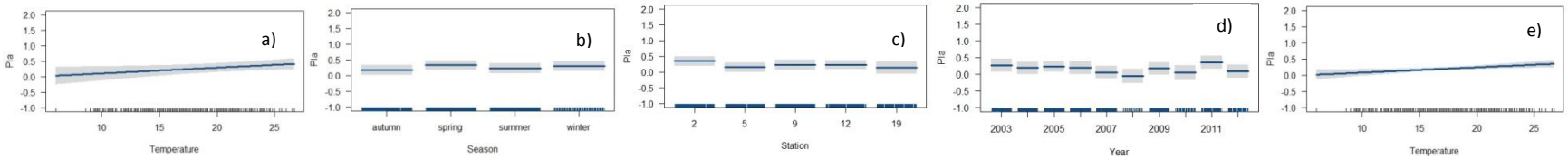


Figure 53 – Response of planktivorous to temperature (GLM with factors) (a), to factors season (b), station (c) and year (d) and to variable temperature (GLM without factors)€

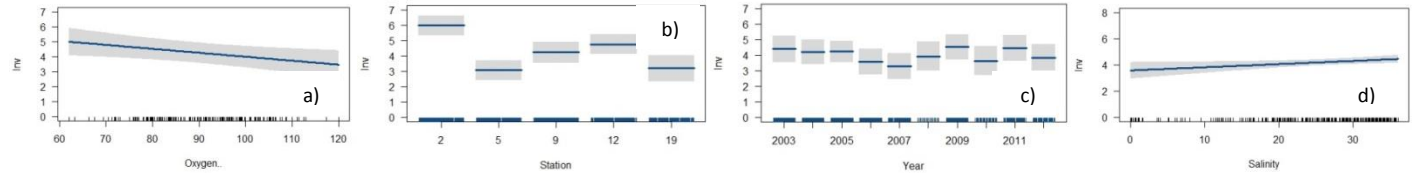


Figure 54– Response of invertebrate feeders to variable oxygen (a), to factors station (b) and year (c) and to variable salinity (d)

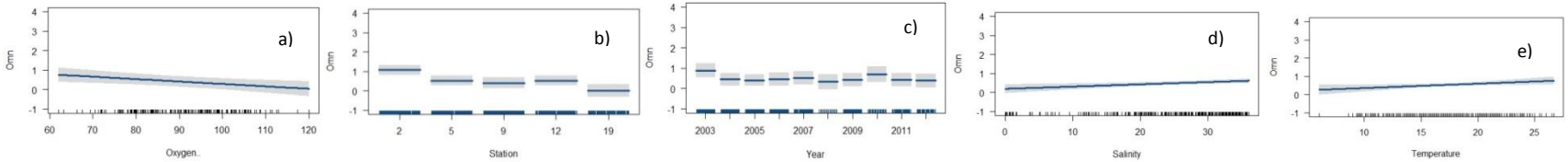


Figure 55 – Response of omnivorous to variable oxygen (a), to factors station (b) and year (c) and to variables salinity (d) and temperature €

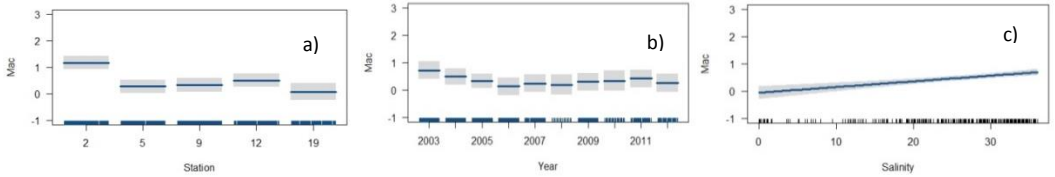


Figure 56– Response of macrocarnivorous to factors station (a) and year (b) and to variable salinity (c)

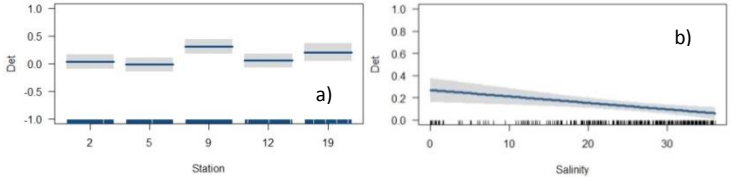


Figure 57 – Response of detritivorous to factor station (a) and to variable salinity (b)

Feeding mode

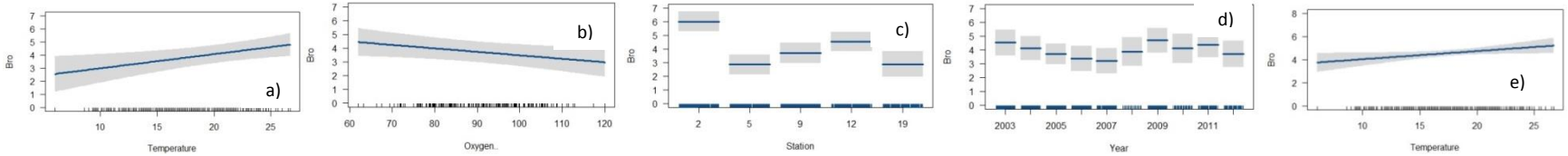


Figure 58 – Response of browsers to variable temperature (GLM with factors) (a) and oxygen (b), to factors station (c) and year (d) and to variable temperature (GLM without factors) (e)

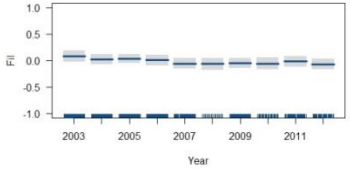


Figure 59 – Response of filterers to factor year

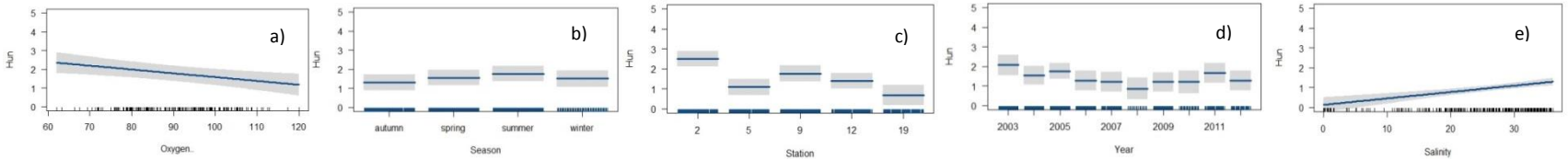


Figure 60 – Response of hunters to variable oxygen (a), to factors season (b), station (c) and year (d) and to variable salinity (e)

Salinity preference

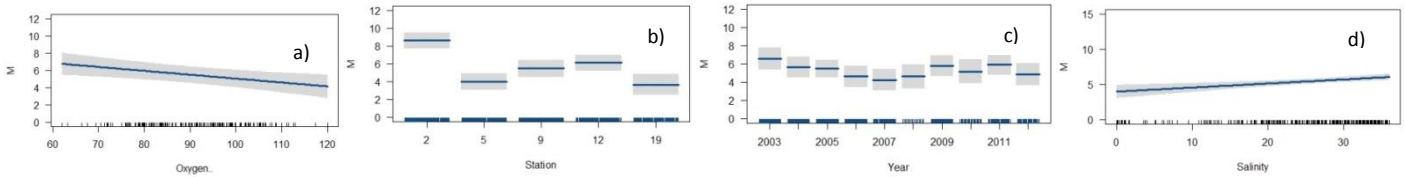


Figure 61 – Response of marine species to variable oxygen (a), to factors station (b) and year (c) and to variable salinity (d)

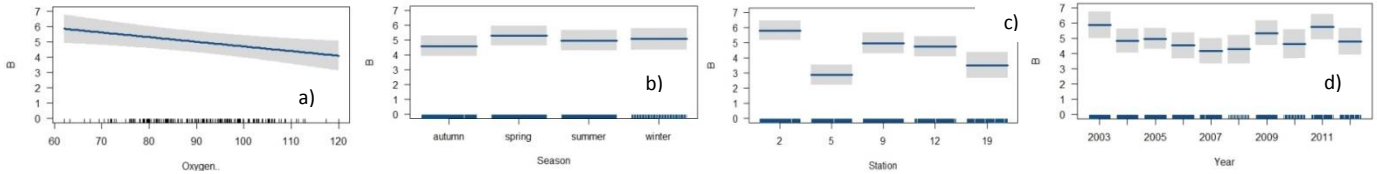


Figure 62 – Response of brackish species to variable oxygen (a) and to factors season (b), station (c) and year (d)

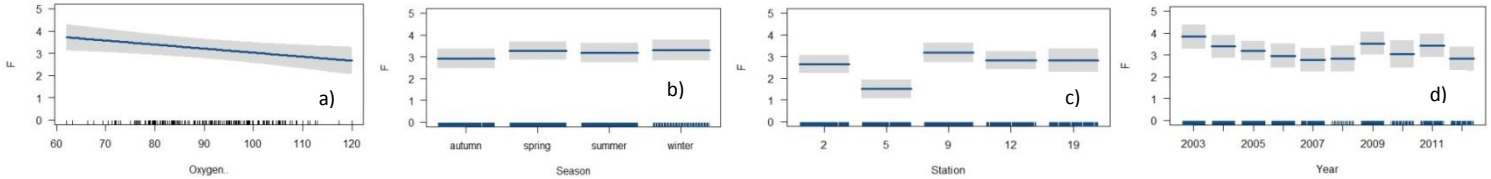


Figure 63 – Response of freshwater species to variable oxygen (GLM with factors) (a) and to factors season (b), station (c) and year (d)

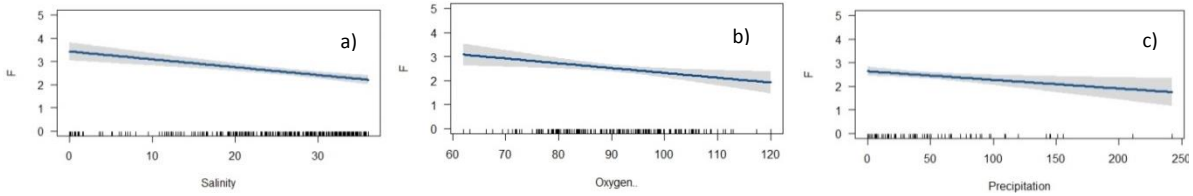


Figure 64 – Response of freshwater species to variables salinity (a), oxygen (GLM without factors) (b) and precipitation (c)